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r-Generalization of Phi Functions For The Subsets Of {m,m+1,…,n}

G.Lalitha[1*] and G.Kamala[2]

[1]SRR&CVR Govt Degree College, Vijayawada, Andhrapradesh, India.
[2]Department of mathematics, Osmania university, Hyderabad, Telangana, India.

Abstract

A nonempty finite set A of positive integers is r-relatively prime if greatest r[th] power common divisor of elements of A is 1. In this case we write $\gcd_r(A)=1$. Let $f^{(r)}(m,n)$ be the number of r-relatively prime subsets of $\{m,\ m+1,\ ...,\ n\}$ and the number of sets in $f^{(r)}(m,n)$ of cardinality k is $f_k^{(r)}(m,n)$. The number of nonempty subsets which are r-relatively prime to n is $\Phi^{(r)}(m,n)$ and the number of sets in $\Phi^{(r)}(m,n)$ of cardinality k is $\Phi_k^{(r)}(m,n)$. We obtained exact formulae and asymptotic estimates for these functions $f^{(r)}(m,n)$, $f_k^{(r)}(m,n)$, $\Phi^{(r)}(m,n)$ and $\Phi_k^{(r)}(m,n)$ in [4]. In this paper we find simple explicit formulae for these four functions which simplify the results in [4] and also find the asymptotic estimates for these functions.

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INTRODUCTION

Let A be a nonempty subset of {1,2,…,n}. The greatest common divisor of elements of A is denoted as $\gcd(A)$. We say that A is relatively prime if $\gcd(A)=1$, and that A is relatively prime to n if $\gcd(A\cup n)=1$. Nathanson [1] defined $f(n)$ is the number of relatively prime subsets of {1,2,…,n} and for $k\geq 1$, $f_k(n)$ is the number of sets in $f(n)$ of cardinality k. The number of nonempty subsets which are relatively prime to n is $\Phi(n)$ and the number of sets in $\Phi(n)$ of cardinality k is $\Phi_k(n)$. M.El.Bachraoui[3] generalized these four functions for the set $\{m,\ m+1,\ ...,\ n\}$. The set A is r-relatively prime if the greatest r[th] power common divisor of elements of A is 1. In this case we write $\gcd_r(A)=1$. The set A is r-relatively prime to n if the greatest r[th] power common divisor of elements of A and n is 1. In this case we write $(\gcd_r(A),n)_r=1$. In [5] we defined the following functions:

$$f^{(r)}(n)=\#\{A\subseteq\{1,2,...,n\}: A\neq\phi,\gcd_r(A)=1\}$$

$$f_k^{(r)}(n)=\#\{A\subseteq\{1,2,...,n\}:\#A=k,\gcd_r(A)=1\}$$

$$\Phi^{(r)}(n)=\#\{A\subseteq\{1,2,...,n\}: A\neq\phi,(\gcd_r(A),n)_r=1\}$$

$$\Phi_k^{(r)}(n)=\#\{A\subseteq\{1,2,...,n\}:\#A=k,(\gcd_r(A),n)_r=1\}$$

and obtained the exact formulae and asymptotic estimates for these functions in [5]. We generalized these four functions for the set $\{m,\ m+1,\ ...,\ n\}$ where $n\geq m$, and obtained exact formulae for the functions $f^{(r)}(m,n)$, $f_k^{(r)}(m,n)$, $\Phi^{(r)}(m,n)$ and $\Phi_k^{(r)}(m,n)$ in [4]. In the present paper we further simplify the exact formulae which are obtained in [4] and find the asymptotic estimates for these four functions.

DEFINITIONS

$$f^{(r)}(m,n)=\#\{A\subseteq\{m,m+1,...,n\}: A\neq\phi,\gcd_r(A)=1\}$$
$$f_k^{(r)}(m,n)=\#\{A\subseteq\{m,m+1,...,n\}:\#A=k,\gcd_r(A)=1\}$$

$$\Phi^{(r)}(m,n)=\#\{A\subseteq\{m,m+1,...,n\}: A\neq\phi,(\gcd_r(A),n)_r=1\}$$

$$\Phi_k^{(r)}(m,n)=\#\{A\subseteq\{m,m+1,...,n\}:\#A=k,(\gcd_r(A),n)_r=1\}$$

We obtain the explicit formulae and asymptotic estimates for these four functions. The following inequality is used.

$$[x]-[y]\leq[\,x-y\,]+1$$

Theorem 1 : Let m,n be non-negative integers. Then for $m < n$,

(i) $$f^{(r)}(m,\ n) = \sum_{1 \le d^r \le n} \mu_r\left(d^r\right)\left(2^{\left\lfloor \frac{n}{d^r} \right\rfloor - \left\lfloor \frac{m-1}{d^r} \right\rfloor} - 1\right)$$

(ii) $$0 \le 2^{n-m+1} - 2^{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor} - f^{(r)}(m,\ n) \le 2n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor}$$

if $\{m,\ m+1,\ ...,\ n\}$ contains multiplies of 2^r, and

$$0 \le -2^{n-m+1} - f^{(r)}(m,\ n) \le 2n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor} + 2^{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}$$

if $\{m,\ m+1,\ ...,\ n\}$ has no multiplies of 2^r.

Proof **: (i)** We have proved in [4], that

$$f^{(r)}(m,\ n) = \sum_{1 \le d^r \le n} \mu_r\left(d^r\right)\left(2^{\left\lfloor \frac{n}{d^r} \right\rfloor} - 1\right) - \sum_{i=1}^{m-1}\left(\sum_{d^r \mid i} \mu_r\left(d^r\right) 2^{\left\lfloor \frac{n}{d^r} \right\rfloor - \left\lfloor \frac{i}{d^r} \right\rfloor}\right)$$

Which can be written as

$$f^{(r)}(m,\ n) = \sum_{1 \le d^r \le n} \mu_r\left(d^r\right)\left(2^{\left\lfloor \frac{n}{d^r} \right\rfloor} - 1\right) - \sum_{1 \le d^r \le m-1} \mu_r\left(d^r\right) 2^{\left\lfloor \frac{n}{d^r} \right\rfloor} \sum_{i=1}^{m-1} 2^{-\frac{i}{d^r}}$$

$$= \sum_{1 \le d^r \le n} \mu_r\left(d^r\right)\left(2^{\left\lfloor \frac{n}{d^r} \right\rfloor} - 1\right) - \sum_{1 \le d^r \le m-1} \mu_r\left(d^r\right) 2^{\left\lfloor \frac{n}{d^r} \right\rfloor}\left(\sum_{j=1}^{\left\lfloor \frac{m-1}{d^r} \right\rfloor} 2^{-j}\right)$$

$$= \sum_{1 \le d^r \le n} \mu_r\left(d^r\right) 2^{\left\lfloor \frac{n}{d^r} \right\rfloor}\left(1 - \sum_{j=1}^{\left\lfloor \frac{m-1}{d^r} \right\rfloor} 2^{-j}\right) - \sum_{1 \le d^r \le n} \mu_r\left(d^r\right)$$

Note that $\left\lfloor \frac{m-1}{d^r} \right\rfloor = 0$ if $m \le d^r \le n$

$$= \sum_{1 \le d^r \le n} \mu_r\left(d^r\right) 2^{\left\lfloor \frac{n}{d^r} \right\rfloor}\left(1 - \left(1 - 2^{-\left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)\right) - \sum_{1 \le d^r \le n} \mu_r\left(d^r\right)$$

$$= \sum_{1\le d^r \le n} \mu_r\left(d^r\right)\left(2^{\left\lfloor \frac{n}{d^r}\right\rfloor - \left\lfloor \frac{m-1}{d^r}\right\rfloor} - 1\right).$$

(ii) Let $1 \le d^r \le n.$ Then $m \le a \le n$ and $d^r \mid a$ if and only if

$$\left\lceil \frac{m}{d^r} \right\rceil \le \frac{a}{d^r} \le \left\lfloor \frac{n}{d^r} \right\rfloor.$$

Which gives that $A \subseteq \{m,\ m+1,\ ...,\ n\}$ and $\gcd_r(A) = d^r$ if and only if

$A^1 = \frac{1}{d^r} * A \subseteq \left\{\left\lceil \frac{m}{d^r} \right\rceil,\ \left\lceil \frac{m}{d^r} \right\rceil + 1,\ ...,\ \left\lfloor \frac{n}{d^r} \right\rfloor\right\}$ and $\gcd_r\left(A^1\right) = 1.$ Therefore

$$2^{n-(m-1)} - 1 = \sum_{1\le d^r \le n} f^{(r)}\left(\left\lceil \frac{m}{d^r} \right\rceil,\ \left\lfloor \frac{n}{d^r} \right\rfloor\right).$$

$$\Rightarrow 2^{n-(m-1)} - 1 = f^{(r)}(m,\ n) + f^{(r)}\left(\left\lceil \frac{m}{2^r} \right\rceil,\ \left\lfloor \frac{n}{2^r} \right\rfloor\right) + \sum_{3\le d^r \le n} f^{(r)}\left(\left\lceil \frac{m}{d^r} \right\rceil,\ \left\lfloor \frac{n}{d^r} \right\rfloor\right)$$

$$\Rightarrow 2^{n-(m-1)} - 1 \le f^{(r)}(m,\ n) + \left(2^{\left\lfloor \frac{n}{2^r}\right\rfloor - \left\lceil \frac{m-1}{2^r}\right\rceil} - 1\right) + \sum_{3\le d^r \le n} 2^{\left(\left\lfloor \frac{n}{d^r}\right\rfloor - \left\lceil \frac{m-1}{d^r}\right\rceil\right)}$$

$$\Rightarrow 2^{n-(m-1)} \le f^{(r)}(m,\ n) + 2^{\left\lfloor \frac{n}{2^r}\right\rfloor - \left\lceil \frac{m-1}{2^r}\right\rceil} + n \cdot 2^{\left\lfloor \frac{n}{3^r}\right\rfloor - \left\lceil \frac{m-1}{3^r}\right\rceil}$$

$$\le f^{(r)}(m,\ n) + 2^{\left\lfloor \frac{n}{2^r}\right\rfloor - \left\lceil \frac{m-1}{2^r}\right\rceil} + n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r}\right\rfloor + 1}$$

Since $\lfloor x \rfloor - \lceil y \rceil \le \lfloor x - y \rfloor + 1.$

$$2^{n-m+1} - 2^{\left\lfloor \frac{n}{2^r}\right\rfloor - \left\lceil \frac{m-1}{2^r}\right\rceil} - 2n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r}\right\rfloor} \le f^{(r)}(m,\ n) \qquad \text{..... (1)}$$

and hence the lower bound for $f^{(r)}(m,\ n)$ is obtained.

The upper bound for $f^{(r)}(m,\ n)$ is obtained as follows :

If the set $\{m,\ m+1,\ ...,\ n\}$ contains multiples of $2^r,$ then

$$f^{(r)}(m,\ n) \le 2^{n-m+1} - 2^{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}$$

$$\Rightarrow 0 \le 2^{n-m+1} - 2^{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor} - f^{(r)}(m,\ n). \qquad \text{..... (2)}$$

From equations (1) and (2)

$$\Rightarrow 0 \le 2^{n-m+1} - 2^{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor} - f^{(r)}(m,\ n) \le 2n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor}$$

If the set $\{m,\ m+1,\ ...,\ n\}$ has no multiples of 2^r, then

$$f^{(r)}(m,\ n) \le 2^{n-m+1}.$$

Hence

$$0 \le 2^{n-m+1} - f^{(r)}(m,\ n) \le 2n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor} + 2^{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}$$

$$\le 2n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor} + 2 \cdot 2^{\left\lfloor \frac{n-m+1}{2^r} \right\rfloor}$$

$$= 2\left\lfloor n \cdot 2^{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor} + 2^{\left\lfloor \frac{n-m+1}{2^r} \right\rfloor} \right\rfloor.$$

Theorem 2 : Let m,n be non-negative integers. Then for $m < n$, $k \ge 1$,

(i) $$f_k^{(r)}(m,\ n) = \sum_{1 \le d^r \le n} \mu_r\left(d^r\right) \binom{\left\lfloor \frac{n}{d^r} \right\rfloor - \left\lfloor \frac{m-1}{d^r} \right\rfloor}{k}$$

(ii) $$0 \le \binom{n-m+1}{k} - \binom{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}{k} - f_k^{(r)}(m,\ n) \le n \binom{\left\lfloor \frac{n-m+1}{2^r} \right\rfloor + 1}{k}$$

if $\{m,\ m+1,\ ...,\ n\}$ contains multiplies of 2^r and

$$0 \le \binom{n-m+1}{k} - f_k^{(r)}(m,\ n) \le n \binom{\left\lfloor \frac{n-m+1}{2^r} \right\rfloor + 1}{k}$$

if $\{m,\ m+1,\ ...,\ n\}$ does not contain multiples of 2^r.

Proof : (i) In [4] we have proved that

$$f_k^{(r)}(m,\ n) = \sum_{1\le d^r \le n} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor}{k} - \sum_{i=1}^{m-1}\sum_{d^r \mid i} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - \frac{i}{d^r}}{k-1}$$

For $K \ge 1$ and $0 \le M \le N$,we have

$$\binom{N}{K} - \sum_{j=1}^{M}\binom{N-j}{K-1} = \binom{N-M}{\mathrm{K}}$$

$$f_k^{(r)}(m,\ n) = \sum_{1\le d^r \le n} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor}{k} - \sum_{1\le d^r \le m-1} \mu_r\left(d^r\right)\sum_{\substack{i=1 \\ d^r \mid i}}^{m-1}\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - \frac{i}{d^r}}{k-1}$$

$$= \sum_{1\le d^r \le n} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor}{k} - \sum_{1\le d^r \le m-1} \mu_r\left(d^r\right)\sum_{j=1}^{\left\lfloor \frac{m-1}{d^r}\right\rfloor}\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - j}{k-1}$$

$$= \sum_{1\le d^r \le m-1} \mu_r\left(d^r\right)\left[\binom{\left\lfloor \frac{n}{d^r}\right\rfloor}{k} - \sum_{j=1}^{\left\lfloor \frac{m-1}{d^r}\right\rfloor}\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - j}{k-1}\right] + \sum_{m\le d^r \le n} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor}{k}$$

$$= \sum_{1\le d^r \le m-1} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - \left\lfloor \frac{m-1}{d^r}\right\rfloor}{k} + \sum_{m\le d^r \le n} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - \left\lfloor \frac{m-1}{d^r}\right\rfloor}{k}$$

Note that $\left\lfloor \frac{m-1}{d^r}\right\rfloor = 0$ if $m \le d^r \le n$.

$$= \sum_{1\le d^r \le n} \mu_r\left(d^r\right)\binom{\left\lfloor \frac{n}{d^r}\right\rfloor - \left\lfloor \frac{m-1}{d^r}\right\rfloor}{k}.$$ Which proves (i).

(ii) The upper bound for $f_k^{(r)}(m,\ n)$ is obtained by deleting k-element sets of multiples of 2^r if they belong to the set $\{m,\ m+1,\ ...,\ n\}$. If the set contains multiples of 2^r, then the upper bound for $f_k^{(r)}(m,\ n)$ is obtained by deleting sets of order k from the set

$$\left\{\left\lfloor \frac{m}{2^r}\right\rfloor,\ \left\lfloor \frac{m+1}{2^r}\right\rfloor,\ ...,\ \left\lfloor \frac{n}{2^r}\right\rfloor\right\}$$

Hence

$$f_k^{(r)}(m,\ n) \le \binom{n-m+1}{k} - \binom{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}{k}$$

The lower bound for $f_k^{(r)}(m,\ n)$ is obtained as follows:

$$\binom{n-m+1}{k} = \sum_{1 \le d^r \le n} f_k^{(r)}\left(\left[\frac{m}{d^r}\right], \left[\frac{n}{d^r}\right]\right)$$

$$\le f_k^{(r)}(m,\ n) + \binom{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}{k} + \sum_{3^r \le d^r \le n} \binom{\left\lfloor \frac{n}{d^r} \right\rfloor - \left\lfloor \frac{m-1}{d^r} \right\rfloor}{k}$$

$$\le f_k^{(r)}(m,\ n) + \binom{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}{k} + n\binom{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor + 1}{k}$$

$$\therefore\ 0 \le \binom{n-m+1}{k} - \binom{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}{k} - f_k^{(r)}(m,\ n) \le n\binom{\left\lfloor \frac{n-m+1}{3^r} \right\rfloor + 1}{k}.$$

If the set $\{m,\ m+1,\ ...,\ n\}$ does not contain multiples of 2^r, then

$$f_k^{(r)}(m,\ n) \le \binom{n-m+1}{k} \Rightarrow\ 0 \le \binom{n-m+1}{k} - f_k^{(r)}(m,\ n).$$

Also

$$\binom{n-m+1}{k} = \sum_{1 \le d^r \le n} f_k^{(r)}\left(\left[\frac{m}{d^r}\right], \left[\frac{n}{d^r}\right]\right)$$

$$= f_k^{(r)}(m,\ n) + \sum_{2 \le d^r \le n} f_k^{(r)}\left(\left[\frac{m}{d^r}\right], \left[\frac{n}{d^r}\right]\right)$$

$$\le f_k^{(r)}(m,\ n) + n\binom{\left\lfloor \frac{n}{2^r} \right\rfloor - \left\lfloor \frac{m-1}{2^r} \right\rfloor}{k}$$

$$\le f_k^{(r)}(m,\ n) + n\binom{\left\lfloor \frac{n-m+1}{2^r} \right\rfloor + 1}{k}$$

$$\therefore\ 0 \le \binom{n-m+1}{k} - f_k^{(r)}(m,\ n) \le n \binom{\left\lfloor \frac{n-m+1}{2^r} \right\rfloor + 1}{k}.$$

Which proves (ii).

***Theorem* 3:** Let m,n be non-negative integers. Then for, $m < n$

(i) $$\Phi^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right) \binom{2^{\frac{n}{d^r}} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}{}$$

(ii) If p is the smallest prime such that $p^r \mid n$, then

$$0 \le 2^{n-m+1} - 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} - \Phi^{(r)}(m,\ n) \le 2n \cdot 2^{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor}.$$

***Proof* : (i)** In [4] we obtained

$$\Phi^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} - \sum_{i=1}^{m-1} \sum_{d^r \mid \gcd_r(i,\ n)} \mu_r\left(d^r\right) 2^{\frac{n-i}{d^r}}.$$

Which can be written as

$$\Phi^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} - \sum_{d^r \mid n} \mu_r\left(d^r\right) \sum_{i=1}^{m-1} 2^{\frac{n-i}{d^r}}$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} - \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} \sum_{i=1}^{m-1} 2^{\frac{-i}{d^r}}$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} - \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} \sum_{j=1}^{\left\lfloor \frac{m-1}{d^r} \right\rfloor} 2^{-j}$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} \left(1 - \sum_{j=1}^{\left\lfloor \frac{m-1}{d^r} \right\rfloor} 2^{-j}\right)$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r}} \left(1 - \left(1 - 2^{-\left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)\right)$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right)\left(2^{\frac{n}{d^r}} \cdot 2^{-\left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right) 2^{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}$$

which proves (i).

(ii) For the smallest prime divisor p of n such that $p^r \mid n$, if we delete all subsets of $\{m,\ m+1,\ \ldots,\ n\}$ whose elements are multiples of p^r, we get

$$\Phi^{(r)}(m,\ n) \le 2^{n-(m-1)} - 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}$$

$$\Rightarrow\ 0 \le 2^{n-(m-1)} - 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} - \Phi^{(r)}(m,\ n).$$

The lower bound for $\Phi^{(r)}(m,\ n)$ can be obtained as follows:

$$\Phi^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right)\left(2^{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)$$

$$= \mu_r(1) 2^{n-(m-1)} + \mu_r\left(p^r\right) 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} + \sum_{\substack{d^r \mid n \\ d > p}} \mu_r\left(d^r\right)\left(2^{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)$$

$$\Phi^{(r)}(m,\ n) = 2^{n-m+1} - 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} + \sum_{\substack{d^r \mid n \\ d > p}} \mu_r\left(d^r\right)\left(2^{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)$$

$$\Phi^{(r)}(m,\ n) - 2^{n-m+1} + 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} = \sum_{\substack{d^r \mid n \\ d > p}} \mu_r\left(d^r\right)\left(2^{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}\right)$$

$$\geq \sum_{\substack{d^r \mid n \\ d>p}} (-1)\, 2^{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}$$

$$\geq (-1)\, n \cdot 2^{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor + 1}$$

$$= -2n \cdot 2^{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor}$$

$$\Rightarrow\ 2^{n-m+1} - 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} - \Phi^{(r)}(m,\ n) \leq 2n \cdot 2^{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor}$$

$$\therefore\ 0 \leq 2^{n-m+1} - 2^{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor} - \Phi^{(r)}(m,\ n) \leq 2n \cdot 2^{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor}.$$

which proves (ii).

Theorem 4 : Let m,n be non-negative integers. Then for $m < n$,

(i) $$\Phi_k^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right) \binom{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}{k}$$

and

(ii) $$0 \leq \binom{n-m+1}{k} - \binom{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}{k} - \Phi_k^{(r)}(m,\ n) \leq n \binom{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor + 1}{k}$$

Proof **: (i)** Let *p* be the smallest prime such that $p^r \mid n$. In [4] , we obtained

$$\Phi_k^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right) \binom{\frac{n}{d^r}}{k} - \sum_{d^r \mid n} \mu_r\left(d^r\right) \sum_{\substack{i=1 \\ d^r \mid i}}^{m-1} \binom{\frac{n-i}{d^r}}{k-1}$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right) \left(\binom{\frac{n}{d^r}}{k} - \sum_{j=1}^{\left\lfloor \frac{m-1}{d^r} \right\rfloor} \binom{\frac{n}{d^r} - j}{k-1} \right)$$

$$= \sum_{d^r \mid n} \mu_r\left(d^r\right)\binom{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}{k}$$

Note that $\binom{N}{K} - \sum_{j=1}^{M}\binom{N-j}{K-1} = \binom{N-M}{\mathrm{K}}$

which proves (i).

(ii) Consider

$$\Phi_k^{(r)}(m,\ n) = \sum_{d^r \mid n} \mu_r\left(d^r\right)\binom{\frac{n}{d^r} - \left\lfloor \frac{m-1}{d^r} \right\rfloor}{k}$$

$$\geq \mu_r(1)\binom{n-(m-1)}{k} - \binom{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}{k} - \sum_{\substack{d^r \mid n \\ d>p}}\binom{\frac{n}{d^r} - \left[\frac{m-1}{d^r}\right]}{k}$$

$$\geq \binom{n-(m-1)}{k} - \binom{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}{k} - \sum_{\substack{d^r \mid n \\ d>p}}\binom{\left[\frac{n-m+1}{d^r}\right]+1}{k}$$

$$\geq \binom{n-(m-1)}{k} - \binom{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}{k} - n\cdot\binom{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor+1}{k}.$$

The upper bound is obtained by deleting k -element sets of $\{m,\ m+1,\ \ldots,\ n\}$ whose elements are multiples of p^r, we get

$$\Phi_k^{(r)}(m,\ n) \leq \binom{n-(m-1)}{k} - \binom{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}{k}.$$

$$\Rightarrow\ 0 \leq \binom{n-(m-1)}{k} - \binom{\frac{n}{p^r} - \left\lfloor \frac{m-1}{p^r} \right\rfloor}{k} - \Phi_k^{(r)}(m,\ n) \leq n\binom{\left\lfloor \frac{n-m+1}{p^r} \right\rfloor+1}{k}$$

which proves (ii).

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Toxicity and Behavioural Studies on the Earthworm, *Lampito mauritii* (Kinberg) Exposed to Organophosphate Insecticide Monocrotophos

M.Ramakrishna[1] , M.VijayaKumar [2],G.Mathew Srirangam[3]
[1,2,3]Assistant Professor, Department of Zoology,
[1]SMBTAV & SN Degree College, Veeravasaram, West Godavari District,
[2]SRR & CVR Government Degree College (A), Vijayawada, Krishna District,
[3]Andhra Loyola College (A), Vijayawada, Krishna District,
Andhra Pradesh, India

Abstract: Earthworms have long been recognized as a 'friend of the farmer'. They plough, aerate and manure the soil ecosystem. Such a defense less friendly creature deserves attention and protection from the onslaught through the irrational use of pesticides. Indiscriminate use of pesticides is bound to disturb the ecological balance of the soil ecosystem. *Lampito mauritii*, a common earthworm in the West Godavari District has been chosen to study the influence of an organophosphate monocrotophos compound which is most commonly used in this area. On calculation the LC_{50} values for 24, 48, 72, 96 hrs were found to be 6.75, 6.25, 5.25 and 4.50 ppm respectively. During the present studies the changes in their morphological features and the patterns of behaviour were observed when exposed to monocrotophos pesticide. The changes were noticed to depend on the experimental periods and concentration levels of the pesticide compounds of the experimental media. The colour of the worms changed from brown to pale brown and mucous material was secreted by the worms. When exposed to monocrotophos the amount of mucous produced was comparatively high at higher concentration and low at low concentration. At higher concentration, *Lampito mauritii* developed swellings, sores and even rupture develop in the entire body when exposed to monocrotophos. Further, protrusion of internal visceral parts through ruptured regions on the body wall became visible in the monocrotophos exposed worms. The data obtained from LC_{50} calculation and the observed behavioural changes were discussed in the light of available literature.
Keywords: *Lampito mauritii,* monocrotophos, LC_{50}, behavioural changes.

INTRODUCTION

One of the major sources of pollution in the terrestrial environment is through indiscriminate and extensive use of biocidal chemicals which are collectively known as pesticides in agricultural operations. It has been now been increasingly realized that the pesticides play an important role in the soil ecosystem. The soil, therefore, acts as a reservoir of pesticides used in the eradication of pests. These pesticides, although primarily aimed at combating pests on crops reach soil system and slowly degrade, gradually spread and possibly get translocated to other environments as well as in the neighborhood through water or air. The rate of degradation of these toxic substances depends upon the constitution of the chemical used. In the above process of dissipation, persistence may result. The pesticides normally become transformed, modified or perhaps magnified through bio-concentrations at different tropic levels in soil ecosystems. Pesticides have been widely used all over the world to control insects, pests and disease vectors and they are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant.

The determination of LC_{50} values is useful in the evaluation of safe level of tolerance of pollutant and moreover it provides fundamental data to design more complex disposal modes of toxicity to the exposed animals. It is suggested that the chronic test, aiming at sublethal effects, is more sensitive and is a more realistic approach for the prediction of environmental effects because in the field, the exposure concentration of pesticides are usually quite low (Rombke *et al*., 2007). Toxicity test are basic tools for ecological risk assessment of toxic compounds. Earthworms are important biocomponents of ecosystem, although not numerically dominant in soil but their large size makes them major contributors to total biomass. They are extremely important in soil formation, principally through their activities in consuming organic matter, fragmenting and mixing it intimately with mineral particles to form aggregates. Pesticides

are either directly applied to the soil to control soil borne pests or deposited on soil as runoff from foliar application. The pesticides residues will impair the physiological functions of earthworms leading to their mortality (Ahmed, 1991). Riepert, (2009) reported that the acute earthworm test is part of the basic test set, but the earthworm reproduction test is considered ecologically more relevant.

Several workers have also investigated the effects of organophosphate insecticides on earthworms' populations. Azinphosmethyl did not affect earthworm populations (Hopkins and Krik, 1957) but carbofuran did (Kring, 1969; Thompson, 1971) Chlorfenvinphos had slight effects (Edwards 1967). Parathion has been reported as moderately toxic to earthworms, particularly in large doses (Heungens, 1966). Senapati (1987) observed the impact of malathion on the population of the earthworms and reported its stressful effect on earthworm in agroecosystem. The earthworms when exposed to organophosphate pesticides showed increased ureotelic and ammonotellic activity (Patnaik and Madhab, 1991). In the same animal Kulkarni (1989) observed that fenvalerate produced pronounced changes in the behavior, and physiology bycausing hormonal and enzymatic imbalance. Goven (1993) have studied the cellular biomarkers for measuring toxicity of xenobiotics. Effect of polychlorinated biphenyls on the coelomocytes of earthworm *Lumbricus terrestris*. From the foregoing account it is clear that the work done on the effects of pesticides on earthworms is scanty. This is especially true in regard to earthworms in India. As mentioned earlier India in general and Andhra Pradesh, in particular, agriculture is the main occupation of a majority of the people.

Lampito mauritii, a common earthworm in the West Godavari District has been chosen to study the influence of one of the pesticide which is most commonly used in the area. The pesticide selected for the present study is organophosphate compound namely, monocrotophos (MCP), commonly known as Azodrin, is an extensively used, potent and highly toxic organophosphate insecticide with and acaricides belonging to the vinyl phosphate group and having a wide range of applications in agriculture (Kavitha and Rao, 2007). This is now in active use in agricultural practices in Lankalakoderu village, Palakol mandal, West Godavari District, Andhra Pradesh in India. *Lampito mauritii*is one of the most common terrestrial oligochaete inhabiting the upper horizon of soils in the southern parts of the Indian sub-continent. Since the use of insecticides has gone up by leaps and bounds now-a-days, it is thought that a study of toxicity of the commonly used organophosphate compounds to *Lampito mauritii* is worthwhile. It is reasonable to expect that the organophosphate compounds even at moderate doses exercise an immediate kill of the earthworm populations. Hence, the present investigations are designed to evaluate the dose-mortality levels of selected organophosphate insecticide namely monocrotophos.

MATERIALS AND METHODS

The earthworm specimens of *Lampito mauritii* were dug-out from the kitchen gardens of residential localities in Lankalakoderu village, Palakol mandal, West Godavari District. They were conveyed to laboratory in wide mouthed plastic jars along with some amount of damp soil collected from their habitat usually within an hour after their collections. After reaching the laboratory they were carefully isolated with a pair of brushes from the soil and gently washed in aerated freshwater. Only healthy, uninjured nearly equal sized worms weighing about 1.0 to 1.5g were selected and acclimated to the laboratory conditions (30 ± 1°C).Since overcrowding causes mortality of the worms, they were maintained in small numbers in batches in number of glass troughs containing fresh water. The media were periodically aerated. The fresh water used for maintenance analyzed to insure that the worms were in a medium of normal composition and therefore under normal physicochemical conditions. They were acclimated for 3 to 4 days to laboratory conditions. They were not fed either during acclimation or experimentation. Lest sudden and or large variations in temperatures should exercise deleterious effects on their survival, both acclimation and the experimentation on the worms were done at constant temperatures. Healthy, active and equal sized worms were chosen for toxicity studies. Standard renewal techniques recommended by (APHA *et al.*, 1998) have been adapted in the present exposure experiments. The renewal techniques followed here were simple and easy to follow in the laboratory.

The media, to which the specific toxicants were added, were freshly prepared. Accumulation excretory products which resulted in deoxygenation and other secondary effects were prevented by frequent renewal of experimental media. Simultaneously control experiments were also made by adding appropriate amounts distilled water to aqueous media. The distilled water added to the control experiments is identical to the quantities used at the maximum toxicant – concentration – exposures. Hence, it was thought fit

simultaneously run controls at the same time. pH and dissolved oxygen content of water media are 7.1 to 7. 5 and 5.6 to 6.5 ml/litre respectively. Preliminary pilot experiments were conducted of exploratory nature to arrive at the broad concentration ranges of pesticides with in which the percentage mortality varied between 5 and 95percent. The concentrations resulting below 5 and above 95 percent mortalities were ignored for the final experiments. The pesticidal concentrations thus selected were used for the final experiments with *Lampito mauritii*. The glassware used was thoroughly cleaned and dried. Filtered and sufficiently aerated pond water was taken in uniform quantities into the various experimental troughs containing worms. Then, appropriate quantities of the stock solution were added to yield the desired concentrations at which the experiments were conducted. The concentration ranges used in the final experiments were in an increasing order at equal intervals of time. Exactly 200ml of water was provided per animal. The toxic media were repeatedly renewed in the experimental chambers as suggested by Environment Protect Agency (EPA, 1975). Periodically the oxygen levels in the experimental media were checked to insure that sufficient oxygen level were maintained.

Immediately after the subjection of the worms to experimental media they were continuously observed hour after hour. Indication of immobility when a touch or mild prick by an entomological pin is given to the posterior part of the worm was considered as dead. The mortalities encountered in each experimental chamber were recorded periodically. In the dose mortality study the total kill observed at the end of each 24hrs period was taken into account for a period of 96hrs. Experiments were conducted in aqueous media to find out LC_{50} values for 24, 48, 72 and 96hrs exposure of the worms to the insecticide monocrotophos. All the experiments were repeated several times to register the constancy of the toxicity of each pesticide. After scoring the mortalities of the worms at various test concentrations, the data were processed employing unweighted regression method as suggested by Finney (1971) to evaluate the LC_{50} (Median Lethal concentrations). The data were recorded and calculated mortalities obtained in these investigations were further subject to heterogeneity test employing chi-square analysis (Finney, 1971). The dose-mortalities obtained in the present study were plotted on a probit graph paper against the calculated dose-mortalities at various concentrations.

RESULTS

The data obtained for *Lampito mauritii* on exposure to organophosphate Insecticide monocrotophos are presented in Table -1. The dose mortalities obtained for *Lampito mauritii* on exposure to 24 hrs to the concentration range of 2.5 to 11.0 ppm of monocrotophos showed 50 percent deaths at a concentration range of 5.90 to 7.59 ppm. On calculation the LC_{50} value for 24hrs obtained was found to be 6.75ppm for monocrotophos, similarly the LC_{50} value for 48hrs was also determined by exposing the worm to a concentration range of 2.0 to 10 ppm of at intervals of 1ppm and the LC_{50} pesticidal concentration for 48hrs was 6.25 ppm. The results indicate that the LC_{50}value for 72 hrs with the concentration range of 1 to 10 ppm was found to be 5.25 ppm. In 96hrs exposure study the pesticide concentration in the test media varied from 0.5 to 9.0ppm at a concentration of 4.50ppm, 50 percent of the test animals died in 96hrs. Hence the LC50 value for 96hrs exposure was found to be 4.50 ppm in Table 1& Fig -1.Concentrations below the LC_{50} concentration level are expressed as sub-lethal concentration. But in the present study a concentration which is 1/5th of the LC_{50} concentration has been considered. The sub- lethal concentration values were 1.35, 1.25, 1.05 and 0.90 ppm for 24, 48, 72 and 96 hrs respectively in Table1 and Fig.1. The results obtained on dose mortality studies of an organophosphate insecticide monocrotophos to *Lampito mauritii* reveal clearly that the worm reacted differently to pesticide concentrations in the present study.

Table 1: Toxicity of monocrotophos to the earth worm *Lampito mauritii*

Exp. Period (hrs)	$^{LC}50$ concentration in ppm	95% Fiducial Limit	1/5th Of $^{LC}50$ Values	95% Fiducial Limit
24	6.75	5.90 -7.59	1.35	1.08-1.62
48	6.25	5.46 -7.03	1.25	1.0-1.50
72	5.25	4.59 -5.90	1.05	0.84-1.26
96	4.50	3.93 -5.06	0.90	0.72-1.08

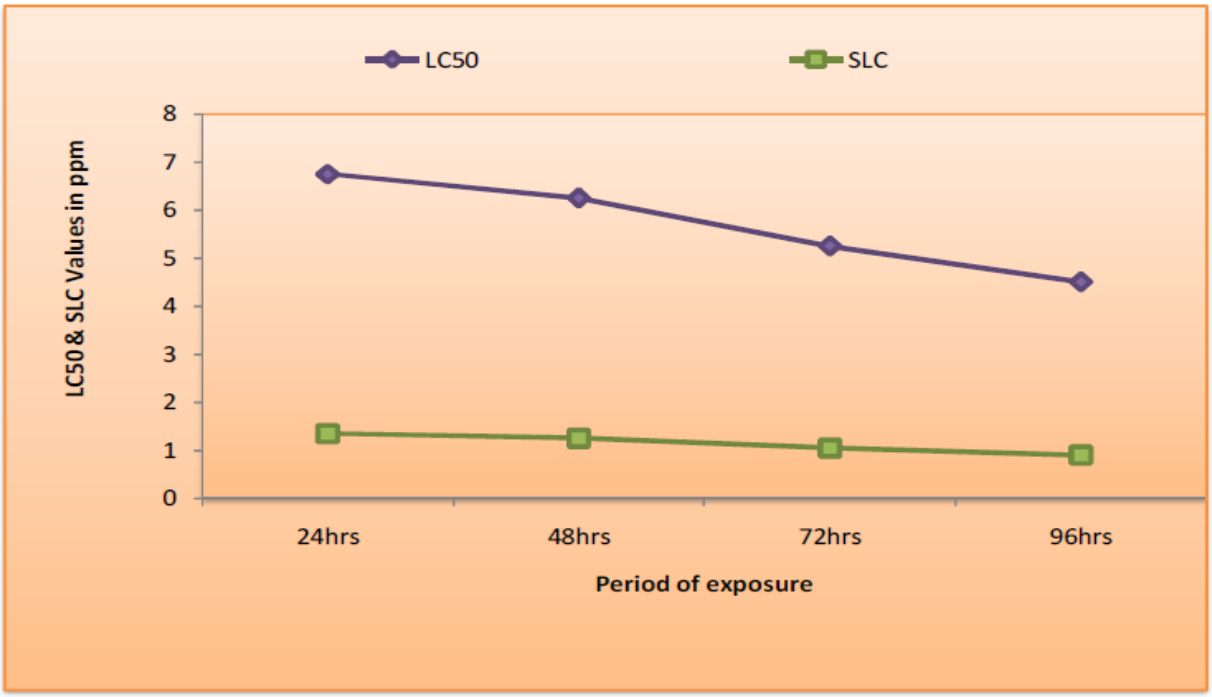

Fig -1: Regression lines showing dose mortality relationship of *L. mauritii* subjected to Monocrotophos for test periods in water medium.

BEHAVIOUR OF WORMS

The present studies revealed the changes in their morphological features and in the patterns of behaviour, when exposed to monocrotophos pesticide. The changes were noticed to depend on the experimental periods and concentration levels of the pesticide compounds of the experimental media. The colour of the worm's changed from brown to pale brown, mucous material was secreted by the worms. When exposed to monocrotophos the amount of mucous produced was comparatively high at higher concentration and low at low concentration. A gradual reduction was noticed in the quantity of mucous secreted with the increase in time of exposure to the pesticide. The intense coiling of the worm noticed after their exposure to toxic media monocrotophos is more effective, as noticed in the present studies, as evidence by the quick and haphazard moments of the worms. After two to three days of exposure the worms became lethargic and moribund and also become immobile and remained curled in a semicircular fashion.

At higher concentration, *Lampito mauritii* developed swellings; sores and even rupture develop in the entire body when exposed to monocrotophos. Further, protrusion of internal visceral parts through ruptured regions on the body wall became visible in the monocrotophos exposed worms (Plate 1-4). In general it was observed that the movement of the worm released in water, containing pesticide, the movements of the worms was invariably quick and erratic in behaviour obviously, as a reaction to toxic action of the chemical. However, this apparent activity of the worms gradually became dissipated with increasing exposure periods of time and concentration.

Moderately large swellings appeared in the anterior parts of the body in the first 3 to 4 segments covering the esophageal region (Plate 1- 4). On certain occasions the worms showed swellings at the clitellar region. The swellings in the posterior segments were less frequently noticed in pesticide exposed worms. It may be mentioned, however, that although observation on the behaviour of the worms indicate the relative levels of toxic influence of the pesticide in the experimental media from the moment they are released into the above media, their movements and/ other changes in the worms noticed at short intervals of time could be made out as it was not possible to quantify the observed differences.

Plate-1: Coiling of the worm

Plate-2: Mucous secretion of the body

Plate-3: Reddening of the entire body

Plate-4: Swelling of the entire body

DISCUSSION

The present studies were principally aimed at investigating the 24, 48, 72 and 96 hrs LC_{50} values on exposure to monocrotophos. The differences between observed and calculated values were tested for significance using chi-square test which showed that the difference was not significant at P = 0.05 level at concentrations 6.75, 6.25, 5.25 and 4.50 ppm respectively. The experimental data clearly indicate the relative toxicity of the insecticide monocrotophos to *Lampito mauritii* under the laboratory conditions.

The results indicate that the worms showed higher mortality rate, even at lower concentrations of monocrotophos high mortality in water may be due to two reasons. The pesticide in medium of water diffuses into body easily through body wall. As there is no food supply, naturally the animal will starve. The test animal being terrestrial animal and soft skinned, possible a quicker exchange of the toxicant is possible and there by neurotoxicity effects may ensure since the quantity of toxicant in water medium may directly enter more readily through the body openings resulting in reddening and in the appearance of swellings in the anterior segments in the worms within two days after their exposure to insecticide (Plates 1 & 2). Similar effects were reported in the case of *Lumbricus terrestris, Lumbricus rubellus, Eisenia foetida, Aporrectodea caligilosa, Allobophora chlorotica, Lampito mauritii* and *Parvularcula bermudensis* when exposed to variety of organophosphate, organochlorine and carbamate insecticides (Vijayalakshmi, 1980; Janardhanarao, 1984). In general it was the movements of the worm released in water containing pesticide, the movements of the worms were invariably quick and erratic in behaviour, obviously as a reaction to toxic action of the chemical. However, this apparent activity of the worms gradually became dissipated with increasing exposure periods of time and concentration. Moderately large swellings appeared in the anterior parts of the body in the first 3 to 4 segments covering the esophageal region. On certain occasions the worms showed swellings at the clitellar region. The swellings in the posterior segments were less frequently noticed in the toxic media exposed worms (Sattibabu, 2013; Rakesh, 2014). Stenersen et.al., (1973) is of the opinion that most of the carbamate insecticides like carbaryl, carbofuran are highly toxic to the earthworms like *Lumbricus terrestris* Cathey, (1973); Kring, (1969); Gilman & Vardanis (1974) on exposure. The pesticides were reported to cause characteristic sores and tumor-like swellings in earthworms. Like organophosphate insecticides and several carbamates were also found to be neurotoxic producing systemic changes in a number of aquatic and terrestrial organisms (Vijayalakshmi, 1980).At higher concentration,

Lampito mauritii developed swelling in the entire body when exposed to dichlorvos. Protrusion of internal visceral parts through ruptured regions on the body wall became visible in the dichlorvos exposed worms.

At times the worms showed swellings at the clitellar region (Bharathi and Subba Rao, 1987). Immediately after exposure to pentachlorophenol *Lampito mauritii* have become highly agitated and slowly curled-up into horse shoe and circular shapes followed by arresting their movements. In course of time the anterior part of the worms turned pale and characteristic wounds and sores appeared on different regions of the body. In higher concentration the worms became rigid similar to that reported in *Lumbricus terrestris* exposed to carbofuran and carbaryl (Stenersen, 1973). The present investigation on the worm involving organophosphate compound showed (Plate 1 & 2) similar changes with slight variations in the characteristic sores, swellings, cuts etc., in the pesticide exposed worms. Although the toxicity levels of the insecticide are different to *Lampito mauritii* the characteristic abnormalities found were nearly similar. The reddening, swellings, sores, cuts, blister, etc., in worms noticed in exposed in test media.

In the light of the present findings of comprehensive assessment of the toxicity levels (LC_{50} values) of insecticide and the effects on the earthworms is of a great value. A perusal of literature dealing with assessment of LC_{50} values of insecticide in respect of earthworms indicate that a quite a large number of publications appeared dealing with the toxicity levels of several agro chemicals based on field studies (Doane, 1962; Edwards *et al*., 1968; Thompson, 1973; Vijayalakshmi, 1980; Bharathi,1983; Sattibabu, 2013; Rakesh, 2014). These reports indicate the toxic nature of a number of organochlorine, organophosphate and carbamate compounds to a variety of non-target organisms including earth worms. Very few investigations are available dealing with the toxicity of pesticides to terrestrial oligochaete species under laboratory conditions (Martin and Wiggans, 1959; Stenersen *et al*., 1973, Chio and Sanborn, 1978; Stenersen, 1979a; Stenersen and Oien, 1980, Vijayalakshmi,1980; Bharathi,1983; Sattibabu, 2013; Rakesh, 2014).

In the case of monocrotophos, concentration levels above 1.0 ppm were noticed to be toxic to the aquatic oligochaete *Tubifex tubifex*. In the present instance *Lampito mauritii* being longer in size tolerated a high concentration of monocrotophos shows LC_{50} value as 0.85 ppm for 96 hrs exposure. Kale and Krishnamoorthy (1979) reported 30 day LC_{50} value for carbaryl compound Sevin as 375 ppm in *Pontoscolex corethrurus*. They reported higher concentrations of the carbamate compounds inhibiting release of castings, resulting loss in weight, survivability and retarded growth. They further felt that higher concentration of these pesticides might as well reduce populations in the ecosystem. Cathey (1973) made a valuable contribution in the field of oligochaete toxicology in his study on the toxic effects of carbamate pesticides to *Lumbricus terrestris*, she reported that a 21 day LC_{50} was 20 µg of carbaryl to *Lumbricus terrestris* in addition a good number of behavioral changes such as withdrawal response i.e., extensive coiling, bodily constrictions, swellings, blisters and reddening of anterior parts consequent to carbaryl treatment were also notice in *Lumbricus terrestris.*

A critical examination of the effects of the chemicals chosen reveals that *Lampito mauritii* exhibited initial hyperactivity at low pesticidal concentrations and developed lethargy at higher concentration of the organophosphate compound. The insecticide presently used is systemic in nature and neurotoxic like any other organophosphates or carbamates as reported by Cathey (1973), Stenersen *et al*., (1973), Stenersen (1979b), Kale and Krishnamurthy (1979), Vijayalakshmi (1980), Bharathi (1983), Sattibabu (2013), Rakesh (2014). Based on laboratory studies, Richards and Cutkomp (1946) and Martin and Wiggans (1959) showed that the DDT in water could survive amounts of at least 1: 10,000. The present findings also showed that at lower concentration the pesticide was stimulatory to the worm rather than inhibitory because the pesticide does act on central nervous system. The hyperactivity at low pesticide concentration and the lethargy developed by the worm at high concentration positively affect changes in population of the worms. Capacities for bioaccumulation and transformation by earthworms on repeated applications of the pesticides have been amply demonstrated (Stenersen *et al*., 1973). In the present study *Lampito mauritii* showed that the toxicity increased with exposure period. This suggests that the toxicity is associated with accumulation of monocrotophos in excess amounts that may be metabolized and prove injurious to the earthworms.

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Synthesis and Anticancer Activity of Amide Derivatives of 1,2-Isoxazole Combined 1,2,4-Thiadiazole

S. Shahinshavali[a], R. Sreenivasulu[b], V. R. Guttikonda[c], D. Kolli[d], and M. V. B. Rao[a]*

[a] *Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, 521001 India*
**e-mail: professormandava@gmail.com*

[b] *Department of Chemistry, University College of Engineering (Autonomous), Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, 533003 India*

[c] *Department of Chemistry, SRR and CVR Government Degree College (Autonomous), Vijayawada, Andhra Pradesh, 520004 India*

[d] *Department of Chemistry, Koneru Lakshmaiah Education Foundation, Green Fields, Vaddeswaram, Andhra Pradesh, 522502 India*

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Abstract—A series of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole are synthesized **11a–11j**. Their chemical structures are confirmed by ^{1}H and ^{13}C NMR, and mass spectra. The products are tested for their anticancer activity against four types of human cancer cell lines, including MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian). Etoposide is used as a positive control. Most of the compounds show good anticancer activity. The compounds **11b, 11c, 11d, 11e, 11g,** and **11j** demonstrate more potent activity than etoposide.

Keywords: Luminespib, Cefozopram, isoxazole, thiadiazole, anticancer activity

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INTRODUCTION

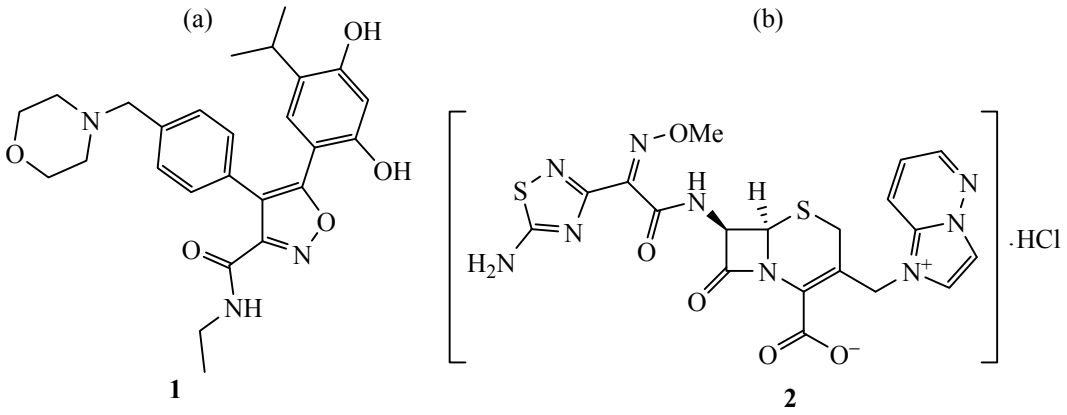
Different types of heterocyclic derivatives are used efficiently in anticancer chemotherapy [1–14]. Isoxazole derivatives are used extensively as agrochemicals and in medicine [15–18] due to a broad spectrum of activity, including anticancer [19], antifungal [20], anti-inflammatory [21], and antimicrobial [22]. Among these, Luminespib (**1**, NVP-AUY922) (see figure) is an FDA approved anticancer drug candidate. Thiadiazole derivatives are important functional components of molecules of many natural compounds [23] and drugs, for example, such as antibiotic Cefozopram (**2**) (see figure) which is used for treatment of CNS [24].

Based on the above information accumulated for isoxazole and thiadiazole and in continuation of our studies of heterocyclic compounds, we designed and synthesized a series of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole **11a**–**11j**. Their structures were confirmed by ^{1}H and ^{13}C NMR and mass spectra. The compounds were tested for anticancer activity against four human cancer cell lines.

RESULTS AND DISCUSSION

Synthetic approach to the target compounds **11a**–**11j** (Scheme 1) started with introduction of compound **3** in the Claisen-Schmidt reaction with 4-cyano-benzaldehyde **4** which led to pure chalcone **5** with good yield. The following reaction of the intermediate **5** with 4-nitrobenzothioamide **6** in presence of $AlCl_3$ gave the product of cycloaddition **7**, which reacted with hydroxylamine hydrochloride to give isoxazole derivative **8**. The following reduction of compound **8** with Zn-dust in acetic acid with formation of amine **9**, and reaction of the latter with aromatic chloroanhydrides **10a**–**10j** led to the title compounds **11a**–**11j**.

Biological evaluation. *In vitro cytotoxicity.* The synthesized compounds **11a**–**11j** were screened for their anticancer activity against four human cancer cell lines such as MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian) by the MTT assay (see the table). Etoposide was used as a positive control. The products **11b**, **11c**, **11d**, **11e**, **11g**, and **11j** displayed higher activity than etoposide. The com-



1 **2**

Structures of (a) Luminespib and (b) Cefozopram.

pounds were examined for structure-activity relationship (SAR). The compound **11b** containing 3,4,5-trimethoxy substituents on the phenyl ring demonstrated high activity with IC_{50} values MCF-7 = 0.24±0.089 μM, A549 = 0.18±0.023 μM, Colo-205 = 0.11±0.05 μM, and A2780 = 0.55±0.072 μM, respectively. Compound **11c** with 3,5-dimethoxy substituents displayed lower activity (MCF-7 = 0.39±0.033, A549 = 1.33±0.45, Colo-205 = 0.93±0.065, and A2780 = 1.37±0.35 μM) than **11b**. The compound **11d** containing one 4-methoxy substituent exhibited the activity lower than the above two analogues. Replacement of 4-methoxy group by 4-chloro substituent on the phenyl ring **11e** resulted in its poorest activity. The compound **11g** with the 4-nitro group exhibited the highest activity. The compound **11h** with 3,5-dinitro substitution exhibited very poor activity. Compounds **11f** and **11i** with the 4-cyano substituent was of moderate activity.

EXPERIMENTAL

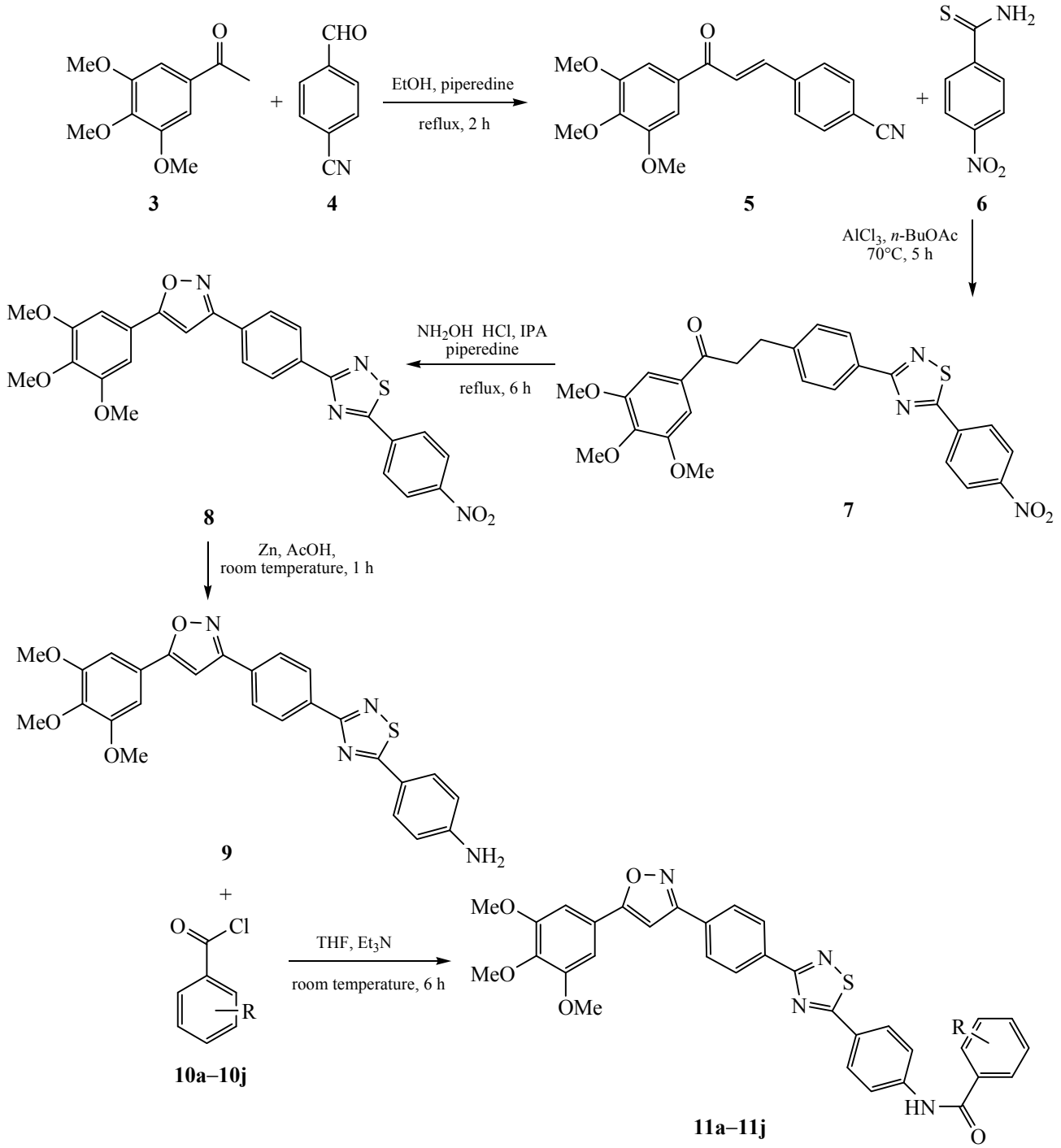
All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualized by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were measured on a Gemini Varian-VXR-unity (300 MHz) spectrometer using DMSO-d_6 as a solvent ($CDCl_3$ for 5) and TMS as the internal standard. ESI spectra were recorded on a Micro mass,

Anticancer activity of the synthesized compounds **11a**–**11j** (IC_{50} μM)[a]

Compound	MCF-7[b]	A549[c]	Colo-205[d]	A2780[e]
11a	2.090±1.87	3.410±1.930	Not active	4.55±2.330
11b	0.240±0.089	0.180±0.023	0.11±0.050	0.55±0.072
11c	0.390±0.033	1.330±0.450	0.93±0.065	1.37±0.350
11d	1.990±0.540	1.830±0.560	1.44±0.880	2.43±1.880
11e	2.440±1.900	1.760±0.190	3.65±1.980	Not active
11f	4.110±2.400	9.670±5.100	Not active	8.34±5.090
11g	0.034±0.004	0.011±0.001	1.23±0.480	0.33±0.022
11h	2.170±1.230	2.880±1.990	7.33±4.100	5.60±4.300
11i	10.40±6.330	3.190±2.150	13.2±7.230	6.23±5.770
11j	1.460±0.320	1.670±0.450	1.42±0.360	Not active
Etoposide	2.110±0.024	3.080±0.135	0.13±0.017	1.31±0.270

[a] Each data is represented as mean ±S.D. of different experiments performed in triplicates. [b] (MCF-7) human breast cancer cell line. [c] (A549) human lung cancer cell line. [d] (Colo-205) human colon cancer cell line. [e] (A2780) human ovarian cancer cell line.

Scheme 1. Synthesis of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole.



R = H (**10a, 11a**), 3,4,5-trimethoxy (**10b, 11b**), 3,5-dimethoxy (**10c, 11c**), 4-methoxy (**10d, 11d**), 4-cloro (**10e, 11e**), 4-bromo (**10f, 11f**), 4-nitro (**10g, 11g**), 3,5-dinitro (**10h, 11h**), 4-cyano, (**10i, 11i**), 4-methyl (**10j, 11j**).

Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined on an electrothermal melting point apparatus, and are uncorrected.

4-[(*E*)-3-(3,4,5-Trimethoxyphenyl)-3-oxoprop-1-enyl]benzonitrile (5). 3,4,5-Trimethoxyacetophenone **3** (20 g, 0.0951 mmol) was dissolved in 50 mL of ethanol, followed by addition of 4-cyanobenzaldehyde

4 (12.5 g, 0.0951 mmol) and 3 drops of piperdine base. The reaction mixture was refluxed for 2 h. After cooling the reaction mixture down, water (20 mL) was added slowly to it. The crystalline precipitate was filtered off and purified by column chromatography using ethyl acetate–hexane (1 : 1) as an eluent to afford pure compound **5**. Yield 73%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 7.28 s (2H), 7.55 d (1H, J = 15.5 Hz), 7.68 d (2H, J = 7.23 Hz), 7.73 d (2H, J = 7.23 Hz), 7.78 d (1H, J = 15.5 Hz). MS (ESI): 325 $[M + H]^+$.

(*E*)-1-(3,4,5-Trimethoxyphenyl)-3-{4-[5-(4-nitrophenyl)-1,2,4-thiadiazol-3-yl]phenyl}prop-2-en-1-one (7). 4-[(*E*)-3-(3,4,5-Trimethoxyphenyl)-3-oxoprop-1-enyl]benzonitrile (**5**) (20 g, 0.0617 mmol) and $AlCl_3$ (8.2 g, 0.0617 mmol) were mixed in *n*-butyl acetate (50 mL), and then, upon stirring at 70°C, 4-nitrobenzothioamide **6** (3.9 mL, 0.030 mmol) was added dropwise. The mixture was stirred at 70°C for 5 h. After cooled down to room temperature and addition of 0.3 mL of water the reaction mixture was stirred at room temperature for 24 h. The organic and aqueous layers were separated, and the aqueous layer was extracted with ethyl acetate (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography on silica gel, using ethyl acetate–petroleum ether (1 : 1) as an eluent to afford pure compound **7**. Yield 70%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 7.28 s (2H), 7.57 d (1H, J = 15.6 Hz), 7.70 d (2H, J = 7.25 Hz), 7.75 d (2H, J = 7.25 Hz), 7.78 d (1H, J = 15.6 Hz), 7.83 d (2H, J = 7.27 Hz), 8.10 d (2H, J = 7.27 Hz). MS (ESI): 505 $[M + H]^+$.

3-{4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]-phenyl}-5-(4-nitrophenyl)-1,2,4-thiadia zole (8). A mixture of compound 7 (20 g, 0.039 mmol) with hydroxylamine hydrochloride (8.2 g, 0.117 mmol) was dissolved in 50 mL of 2-propanol, then 3 mL of pyridine were added and the reaction mixture was stirred upon refluxing for 6 h. After completion of reaction, according to TLC, the solvent was evaporated under reduced pressure. The precipitated product was washed with water (3×20 mL) and purified by column chromatography using ethyl acetate–hexane (7 : 3) as an eluent to afford pure compound **8**. Yield 70%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.66 s (1H), 7.27 s (2H), 7.71 d (2H, J = 7.26 Hz), 7.77 d (2H, J = 7.26 Hz), 7.82 d (2H, J = 7.28 Hz), 8.11 d (2H, J = 7.28 Hz). MS (ESI): 518 $[M + H]^+$.

4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}benzenamine (9). To a solution of (*E*)-1-(3,4,5-trimethoxyphenyl)-3-{4-[5-(4-nitrophenyl)-1,2,4-thiadiazol-3-yl]phenyl}prop-2-en-1-one (**8**) (13 g, 0.0251 mmol) in acetic acid (40 mL) was added zinc powder (2.3 g, 0.0751 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the process, according to TLC, the reaction mixture was filtered (Celite), and the filtrate was evaporated to dryness giving pure compound **9**. Yield 76%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 5.51 br.s (2H), 6.66 s (1H), 7.28 s (2H), 7.70 d (2H, J = 7.25 Hz), 7.76 d (2H, J = 7.25 Hz), 7.80 d (2H, J = 7.27 Hz), 8.09 d (2H, J = 7.27 Hz). MS (ESI): 488 $[M + H]^+$.

General method of synthesis of amide derivatives 11a–11j. The compound 9 (500 mg, 0.0010 mmol) was dissolved in 10 mL of dry THF, and 0.0010 mmol of one of benzoyl chlorides **10a**–**10j** and 0.002 mmol of Et_3N were added. The reaction mixture was stirred at room temperature for 6 h, till completion of the process (TLC), then it was washed with water and extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography using ethyl acetate–hexane (1 : 1) as an eluent to obtain the corresponding pure compound **11a**–**11j**.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)benzamide (11a).** Yield 51%, mp 300–302°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.66 s (1H), 7.28 s (2H), 7.52 t (1H), 7.56–7.66 m (2H), 7.70 d (2H, J = 7.24 Hz), 7.75 d (2H, J = 7.24 Hz), 7.78–7.88 m (4H), 8.07 d (2H, J = 7.26 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.8, 96.4, 106.3, 123.8, 126.5, 128.3, 129.4, 129.7, 130.4, 131.2, 132.7, 133.4, 134.7, 140.5, 145.3, 156.7, 158.6, 159.5, 160.7, 168.4, 170.4. MS (ESI): 592 $[M + H]^+$.

3,4,5-Trimethoxy-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}-phenyl)benzamide (11b). Yield 47%, mp 317–319°C. ^{1}H NMR spectrum, δ, ppm: 3.87 s (6H), 3.90 s (3H), 3.93 s (3H), 3.96 s (6H), 6.65 s (1H), 7.27 s (2H), 7.32 s (2H), 7.69 d (2H, J = 7.23 Hz), 7.76 d (2H, J = 7.23 Hz), 7.80 d (2H, J = 7.25 Hz), 8.08 d (2H, J = 7.25 Hz), 8.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 56.5, 57.8, 61.2, 62.5, 96.3, 106.4, 107.8, 123.4, 126.5, 127.6, 129.8, 130.4, 130.8, 131.5, 133.6, 134.6, 139.6, 143.2, 145.6, 156.3, 157.8, 158.2, 160.5, 163.2, 169.3, 170.6. MS (ESI): 682 $[M + H]^+$.

3,5-Dimethoxy-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)benzamide (11c). Yield 62%, mp 312–314°C. ^{1}H NMR spectrum, δ, ppm: 3.77 s (6H), 3.92 s (3H), 3.95 s (6H), 6.66 s (1H), 7.27 s (2H), 7.30 s (2H), 7.68 d (2H, *J* = 7.24 Hz), 7.77 d (2H, *J* = 7.24 Hz), 7.81 d (2H, *J* = 7.26 Hz), 8.09 d (2H, *J* = 7.26 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 56.4, 57.3, 61.9, 96.4, 106.3, 107.4, 118.4, 123.4, 126.7, 127.4, 129.6, 130.4, 130.8, 133.5, 134.5, 135.2, 139.6, 145.6, 156.4, 158.6, 160.4, 161.5, 164.7, 169.5, 170.7. MS (ESI): 652 $[M + H]^+$.

4-Methoxy-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11d). Yield 59%, mp 307–309°C. ^{1}H NMR spectrum, δ, ppm: 3.79 s (3H), 3.93 s (3H), 3.95 s (6H), 6.65 s (1H), 7.27 s (2H), 7.55 d (2H, *J* = 7.20 Hz), 7.69 d (2H, *J* = 7.25 Hz), 7.73–7.80 m (4H), 7.84 d (2H, *J* = 7.27 Hz), 8.09 d (2H, *J* = 7.27 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 58.3, 61.8, 96.4, 106.3, 115.7, 123.4, 126.5, 128.6, 129.5, 130.4, 130.7, 131.6, 132.4, 133.6, 134.2, 140.7, 145.6, 156.3, 158.7, 160.3, 161.5, 164.6, 169.6, 170.8. MS (ESI): 622 $[M + H]^+$.

4-Chloro-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11e). Yield 70%, mp 288–290°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.95 s (6H), 6.66 s (1H), 7.27 s (2H), 7.63 d (2H, *J* = 7.30 Hz), 7.68 d (2H, *J* = 7.26 Hz), 7.70–7.81 m (4H), 7.86 d (2H, *J* = 7.28 Hz), 8.10 d (2H, *J* = 7.28 Hz), 8.58 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 96.4, 106.4, 123.7, 126.4, 127.6, 129.7, 130.6, 131.3, 132.7, 133.4, 134.2, 134.7, 135.2, 140.3, 140.7, 145.4, 156.4, 158.3, 160.5, 161.6, 169.6, 170.7. MS (ESI): 626 $[M + H]^+$.

4-Bromo-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11f). Yield 66%, mp 276–278°C. ^{1}H NMR spectrum, δ, ppm: 3.92 s (3H), 3.96 s (6H), 6.67 s (1H), 7.26 s (2H), 7.65 d (2H, *J* = 7.31 Hz), 7.69 d (2H, *J* = 7.27 Hz), 7.72–7.83 m (4H), 7.87 d (2H, *J* = 7.29 Hz), 8.10 d (2H, *J* = 7.29 Hz), 8.58 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.3, 61.8, 96.5, 106.4, 123.4, 124.6, 126.5, 129.5, 130.3, 130.8, 131.4, 131.7, 133.4, 134.7, 135.4, 135.8, 140.4, 145.6, 156.3, 158.3, 160.4, 162.8, 168.9, 170.7. MS (ESI): 671 $[M + H]^+$.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl]phenyl)-4-nitro-benzamide (11g).** Yield 73%, mp 283–285°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.67 s (1H), 7.27 s (2H), 7.69 d (2H, *J* = 7.28 Hz), 7.73 d (2H, *J* = 7.28 Hz), 7.84–7.95 m (4H), 8.11 d (2H, *J* = 7.30 Hz), 8.20 d (2H, *J* = 7.32 Hz), 8.59 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.8, 96.7, 106.4, 123.4, 125.7, 126.5, 127.5, 129.7, 130.3, 130.8, 131.4, 133.6, 134.6, 136.5, 140.4, 145.6, 151.3, 156.7, 158.4, 160.5, 161.7, 169.5, 170.7. MS (ESI): 637 $[M + H]^+$.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl)phenyl)-1,2,4-thiadiazol-5-yl)phenyl)-3,5-dinitro-benzamide (11h).** Yield 71%, mp 309–311°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.68 s (1H), 7.27 s (2H), 7.70 d (2H, *J* = 7.28 Hz), 7.74 d (2H, *J* = 7.28 Hz), 7.83 d (2H, *J* = 7.31 Hz), 8.12 d (2H, *J* = 7.31 Hz), 8.30 s (1H), 8.36 s (2H), 8.59 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.9, 96.7, 106.8, 123.5, 124.7, 126.7, 128.4, 129.6, 130.7, 131.3, 131.7, 133.4, 134.5, 135.2, 136.5, 145.6, 148.3, 156.5, 158.6, 159.3, 160.4, 169.8, 171.8. MS (ESI): 682 $[M + H]^+$.

4-Cyano-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11i). Yield 82%, mp 269–271°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.68 s (1H), 7.27 s (2H), 7.68 d (2H, *J* = 7.26 Hz), 7.72 d (2H, *J* = 7.26 Hz), 7.81–7.94 m (4H), 8.10 d (2H, *J* = 7.28 Hz), 8.18 d (2H, *J* = 7.27 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 96.5, 106.8, 114.5, 119.6, 123.5, 126.8, 129.7, 130.3, 130.7, 131.4, 131.9, 133.5, 134.5, 135.7, 139.4, 140.3, 145.5, 156.4, 158.6, 160.7, 163.4, 169.8, 170.8. MS (ESI): 617 $[M + H]^+$.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-4-methyl-benzamide (11j).** Yield 54%, mp 265–267°C. ^{1}H NMR spectrum, δ, ppm: 2.43 s (3H), 3.93 s (3H), 3.96 s (6H), 6.66 s (1H), 7.24 s (2H), 7.46 d (2H, *J* = 7.19 Hz), 7.55 d (2H, *J* = 7.19 Hz), 7.68 d (2H, *J* = 7.23 Hz), 7.72 d (2H, *J* = 7.23 Hz), 7.80 d (2H, *J* = 7.26 Hz), 8.09 d (2H, *J* = 7.26 Hz), 8.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 24.8, 57.6, 61.8, 96.4, 106.8, 123.4, 126.5, 128.5, 129.6, 130.4, 131.3, 131.7, 132.4, 133.5, 134.2, 135.4, 140.6, 143.5, 145.6, 156.7, 158.6, 160.4, 163.6, 169.7, 170.9. MS (ESI): 606 $[M + H]^+$.

MTT assay. Individual wells of a 96-well tissue culture microtiter plate were inoculated with 100 μL of complete medium containing 1×10^4 cells. The plates were incubated at 37°C in a humidified 5% CO_2 incubator for 18 h prior to the experiment. After medium removal, 100 μL of fresh medium containing the test compounds and etoposide at different

concentrations (0.5, 1, 2 μM) were added to each well and incubated at 37°C for 24 h. The medium was discarded and replaced with 10 μL MTT dye. Plates were incubated at 37°C for 2 h. The resulting formazan crystals were solubilized in 100 μL extraction buffer. Optical density (O.D.) was measured at 570 nm with a micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

CONCLUSIONS

A number of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole **11a**–**11j** is synthesized. All the compounds are tested for their anticancer activity against four types of human cancer cell lines including MCF-7 (breast), A549 (lung), Colo-205 (colon) and A2780 (ovarian). Most of the compounds demonstrate significant anticancer activity, and **11b**, **11c**, **11d**, **11e**, **11g**, and **11j** exhibit more potent activity than etoposide. The compound **11g** demonstrates the superior activity.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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A Validated Reversed Phase HPLC Method Development for the Assay of Ciprofloxacin in Oral Suspension

Bathula Sivannarayana[1], Modugula Jyosthna[1], Dr Dasi Jeevan Mani Babu[1], Mekala Sunil[1], Choppavarapu Dorcas Anand[2], Dr M. Manoranjani[3]

[1]St.Xavier Institute of Pharmacy, Phirangipuram, Guntur. A.P., India
[2]SRR & CVR Govt. (A) Degree College, Vijayawada, A.P., India
[3]Dept. of Chemistry, PB Siddartha College of Arts and Sciences, Vijayawada, A.P., India

E-Mail: drjeevanbabu@gmail.com

Keywords:
Ciprofloxacin, RP-HPLC

ABSTRACT

A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for the determination of Ciprofloxacin in oral suspension. The separation was carried out using a mobile phase consisting of buffer of pH 2.0 and Acetonitrile in the ratio of 87: 13. The column used was Inertsil ODS-3 4.6×250mm, 5µ. with a flow rate of 1.5 ml / min by detection at 278 nm. The described method was linear over a concentration range of 25-150%. The retention time of Ciprofloxacinwas found to be 9.4min. Results of analysis were validated statistically and by recovery studies. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ciprofloxacinin its pharmaceutical dosage forms.

1. INTRODUCTION

Ciprofloxacin is a broad-spectrum antimicrobial carboxyfluoroquinoline. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercooling repair, and recombination. [1]

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Ciprofloxacin is a broad-spectrum anti-infective agent of the fluoroquinolone class. Ciprofloxacin has in vitro activity against a wide range of gram-negative and gram-positive microorganisms. The mechanism of action of quinolones, including ciprofloxacin, is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to ciprofloxacin. There is no known cross-resistance between ciprofloxacin and other classes of antimicrobials. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercooling repair, and recombination.

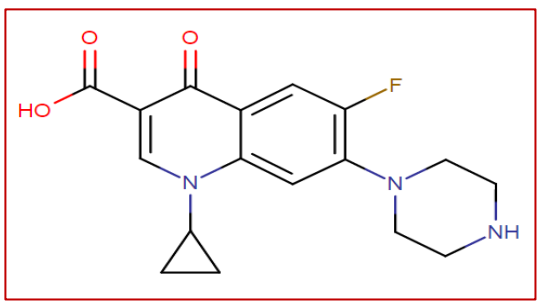

Figure. 1. Molecular structure of Ciprofloxacin

2. Materials and Methods:

2.1 Chemicals and Reagents:

Standard bulk drug sample Ciprofloxacin was provided by Chandra labs, Hyderabad. All the chemicals used were of analytical and HPLC grade procured from Qualigens, India Ltd. The chemicals used for this study were Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade), Ortho phosphoric acid (Analytical grade). Waters HPLC 2695with UV detector was used for the analysis.

2.2. Preparation of Mobile Phase:

Mobile phase-A: (Buffer) Pipette out 10ml of Methanol in to 1000ml of and mix. Adjust to pH 2.0 with Orthophosphoric acid, then filter through 0.45µ filter paper and sonicate for 2minutes.

Mobile phase-B: Acetonitrile

Preparation of Mobile Phase:

Mix the mobile phase-A and mobile phase-B in the ratio in the ratio 87:13%v/v.

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2.3. Preparation of Stock and Standard Solutions:

Preparation of Standard solution:

Weigh and transfer 25mg ciprofloxacin working standard into a 50mL volumetric flask. Add 7ml of acetonitrile andsonicate for 2minutes then add 30ml of pH2.00buffer, sonicate for 10minutes then make up to the mark with diluent. Further pipette out 5ml of the above solution in to 20ml volumetric flask, add 2.8ml of acetonitrile mix well, then add 12ml of pH2.00 buffer, then make up to the mark with diluent.

Preparation of Test Solution:

Shake the bottle 10minutes immediately prior to sampling in order to accomplish homogeneity of suspension. Weigh and transfer 5.5g ciprofloxacin suspension into a 500mL volumetric flask. Add 70ml of acetonitrile sonicate for 10 minutes, then add 250ml of pH 2.00 buffer, sonicate for 20minutes then make up to the mark with diluent. Further pipette out 5ml of the above solution in to 20ml volumetric flask, add 2.8ml of acetonitrile mix well, then add 12ml of pH2.00 buffer, then make up to the mark with diluent.

2.4. Optimized Chromatographic Conditions:

Column: Inertsil ODS-3 4.6×250mm, 5µ.

Flow rate : 1.5 mL /min.

Wavelength : 278 nm

Column temperature : 40°C

Injection Volume : 10 µL

Run Time : 15 minutes

Retention time: Ciprofloxacin, RT about 9.4min

3. Method Validation Parameters:

Linearity:

A series of Ciprofloxacin solutions were prepared in the concentration ranging from 25% to 150% of specification level and injected into the HPLC system as per the test method. The square of the correlation coefficient, intercept and residual sum of squares were calculated.

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Accuracy:

A series of solutionswereprepared in triplicatetest preparation at the specification limit in the range of about 25%to 150% of test concentration and injected into HPLC system and analyzed as per the test method. Individual % recovery, mean % recovery, %RSD and linearity of the testmethodwere calculated at each level.

Intermediate Precision:

To evaluate the intermediate precision for assay method, six samples were prepared and analyzed as per test method by using different column, by different analysts on different days. Intermediate precision was calculated and found to be within the acceptable limits. The overall % RSD of six samples in method precision, intermediate precision (n=6 and n=12) were calculated.

Filter Validation:

A study was conducted to evaluate the filter suitability by using two different types of filters namely0. 45 µm PVDF and 0.45µm Nylon filters. Standard solution was prepared in single and test solution was prepared in duplicate as per the test method. Portion of standard and test solutions were filtered through 0.45 µm PVDF, 0.45 µm nylon filter and some portion of standard and sample solutions were centrifuged and analyzed as per test method.

Robustness:

Flow Rate Variation:

A study was conducted to determine the effect of variation in flow rate. Blank, Standard and sample (at the specification level) were prepared as per the test method and injected into the HPLC system with flow rates of 1.4ml/minute and 1.6ml/minute. The system suitability parameters sample was evaluated and found to be within the specified limits as per test method.

Column Oven Temperature Variation:

A study was conducted to determine the effect of variation in Column oven Temperature. Standard and test preparations (at the specification level) were prepared as per the test method and injected into the HPLC system with a column oven temperature of 35°C and 45°C. System suitability parameters and sample were evaluated and found to be within the specified limits as per test method.

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Effect of Variation In Mobile Phase Composition:

A study was conducted to determine the effect of variation in mobile phase composition. Two different mobile phases of Buffer and Acetonitrile were prepared in the ratio of 855:145%v/v and 885:115%v/v as per the test method. Standard and test preparations with specification level were prepared as per the test method and injected into the HPLC system.

Effect of pH Variation in Mobile Phase:

A study was conducted to determine the effect of variation in pH in the mobile phase. Two mobile phases of pH 2.80 and 3.20 were prepared as per the test method. Blank, Standard and test preparations were prepared as per the test method and injected into the HPLC system with System suitability parameters and sample were evaluated and found to be within the specified limits as per test method.

The Effect of Wavelength Variation:

A study was conducted to determine the effect of variation in wavelength. Standard and test preparations (at the specification level) were prepared as per the test method and injected into the HPLC system with wavelength of -Ciprofloxacin280nm and 276nm.System suitability parameters and sample were evaluated and found to be within the specified limits as per test method.

4. Results and Discussion:

The solution of Ciprofloxacin was scanned in the range of 200-400nm and 278nm was selected as detection wavelength by RP-HPLC method with an isocratic elution technique. The optimization was done by changing the composition of the mobile phase, ratio and flow rate. Finally the mobile phase with buffer (pH 2): ACN in the ratio 87:13v/v% was optimized for the estimation of Ciprofloxacin and the column used for separation is Inertsil ODS-3 4.6×250mm,5µ. [2]

The chromatographicparameters of system suitability such as %RSD, standard recovery, Tailing factor, Theoretical plates were found to be satisfactory. The values of these parameters are tabulated in Table-1.

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Table.1. System suitability data for Ciprofloxacin

System suitability parameters for Ciprofloxacin	Method Precision	Intermediate precision	Acceptance Criteria
%RSD	0.3	0.3	Not more than 2.0
Standard recovery (%)	101.4	99.5	Between 98.0 to 102.0
Tailing factor	1.1	1.1	Not more than 2.0
Theoretical plates	9925	9271	Not less than 2000

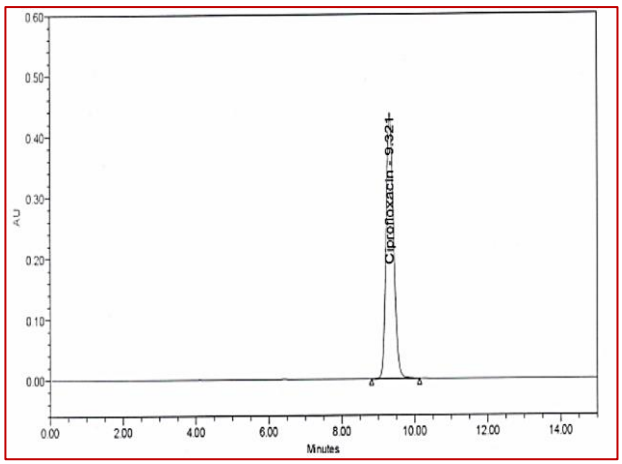

Figure.2.Typical chromatogram of Standard solution

The linearity of the developed method was determined by analyzing different concentrations of the standard solution containing a concentration range from 25% to 150%. The response factor of the standard solutions was calculated. The ratio of peak areas of ciprofloxacin was plotted against the concentration to obtain the calibration graph (Fig. 3) and was found to be linear over the concentration range from 25% to 150%. The data were analyzed by linear regression, least-squares method and the corresponding equation are given by Y = BX + c, where 'Y' is the ratio of the peak areas values of Ciprofloxacin, 'b' is the slope, 'c' is the intercept and 'X' is the concentration of the analyte. Linear regression, least squares fit data are given in (Table 2). [3] The percentage purity was found to be 99.3%. The precision of the method was confirmed by the repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies and the data was given by (Table 3). [4] Similarity factors were calculated for the filtered standards against unfiltered standard (Centrifuged) and found to be within the specified limit. The difference in the % between unfiltered (centrifuged) and filtered samples were calculated and found to be

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meeting the acceptable limit. Both PVDF and Nylon filters were suitable for the intended purpose.

Table.2.Linearity of detector response for Ciprofloxacin

% Linearity level	Concentration (ppm)	Response	Acceptance criteria
25	31.0875	1536480	Square of Correlation co-efficient should not be less than 0.999
50	62.175	3107355	
75	93.2625	4623963	
100	124.35	6039873	
150	186.525	9110398	
Square of correlation coefficient : 0.999 Slope: 48483.76574 Intercept : 60448.78378 Residual sum of squares: 45710.56736			

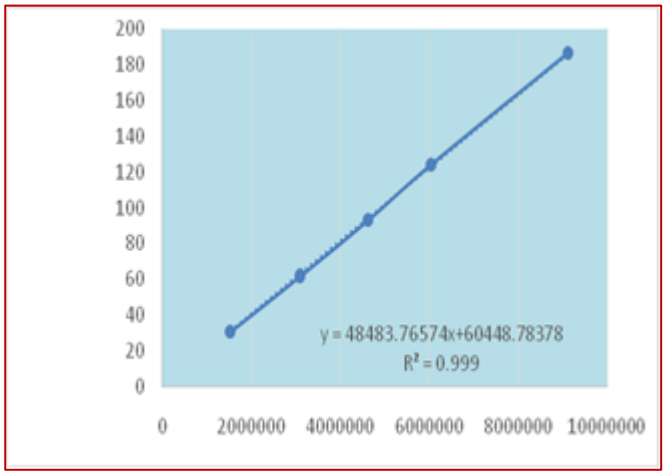

Figure.3.Linearity of detector response graph for Ciprofloxacin.

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Table.3.Accuracy data of Ciprofloxacin

S. No.	% spike level	Amount added (%w/w)	Amount recovered (%w/w)	% Recovery	% Mean recovery	% RSD
1.	25%	62.1229	62.37308	100.4	99.8	0.6
2.		63.0287	62.40228	99.0		
3.		63.0885	62.62924	99.3		
4.		63.1979	62.72669	99.3		
5.		62.2424	62.36469	100.2		
6.		62.3220	62.55297	100.4		
1.	100%	250.8408	247.83270	98.8	99.3	0.4
2.		248.9894	247.87577	99.6		
3.		249.1088	247.78317	99.5		
1.	150%	378.5307	377.14133	99.6	100.6	0.5
2.		374.4894	377.08205	100.7		
3.		374.7781	377.28255	100.7		
4.		373.8921	377.45520	101.0		
5.		373.2252	376.76809	101.0		
6.		374.1609	376.10577	100.5		

Specificity:

Chromatogram of blank and placebo should not show any peak at the retention time of Ciprofloxacin peak and known impurity peaks.

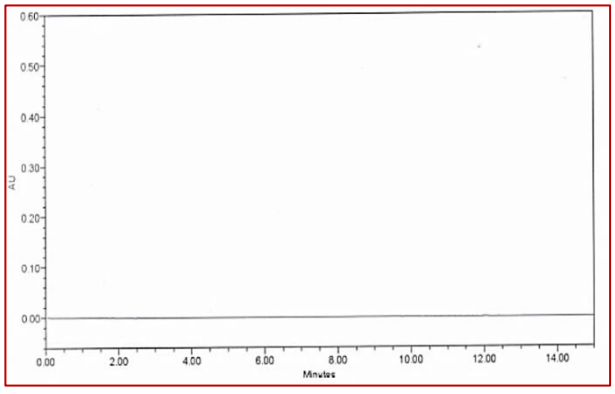

Figure.4.Typical chromatogram of Blank

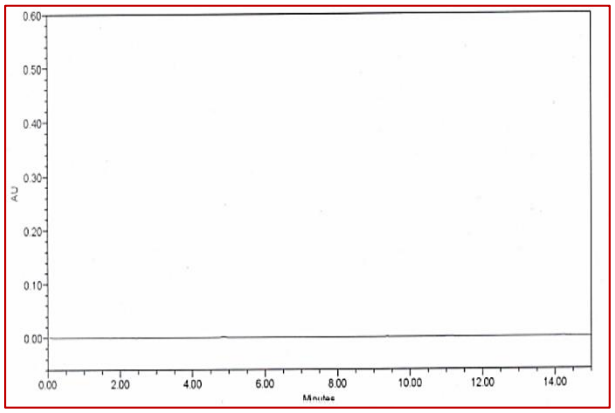

Figure.5.Typical chromatogram of placebo

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Table.4.Method Precision data for Ciprofloxacin

Sample No.	Ciprofloxacin content (%)
1	100.9
2	100.2
3	100.0
4	99.4
5	101.1
6	100.5
Mean	**100.4**
% RSD	**0.6**

5. Conclusion:

This study showed that the antibiotic drug, Ciprofloxacin can be precisely and accurately determined in pure and pharmaceutical dosages. The proposed method is simple and requires less time for analysis. System performance parameters revealed that the method is ideal for the assay ofCiprofloxacin.

Hence, the developed chromatography method was applied for routine analysis and can be used for the intended purpose.

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Indian Journal of Research in Pharmacy and Biotechnology (IJRPB)
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S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry

Training Program

CERTIFICATE COURSE IN MEDICAL CODING

Date: 10/12/2018 to 10/01/2019

Students participated: II, III BSc- MBC, BZC Program students

Resource person: Trainer from VAWE Institute of Technical Training Mrs. P. Swathi

---

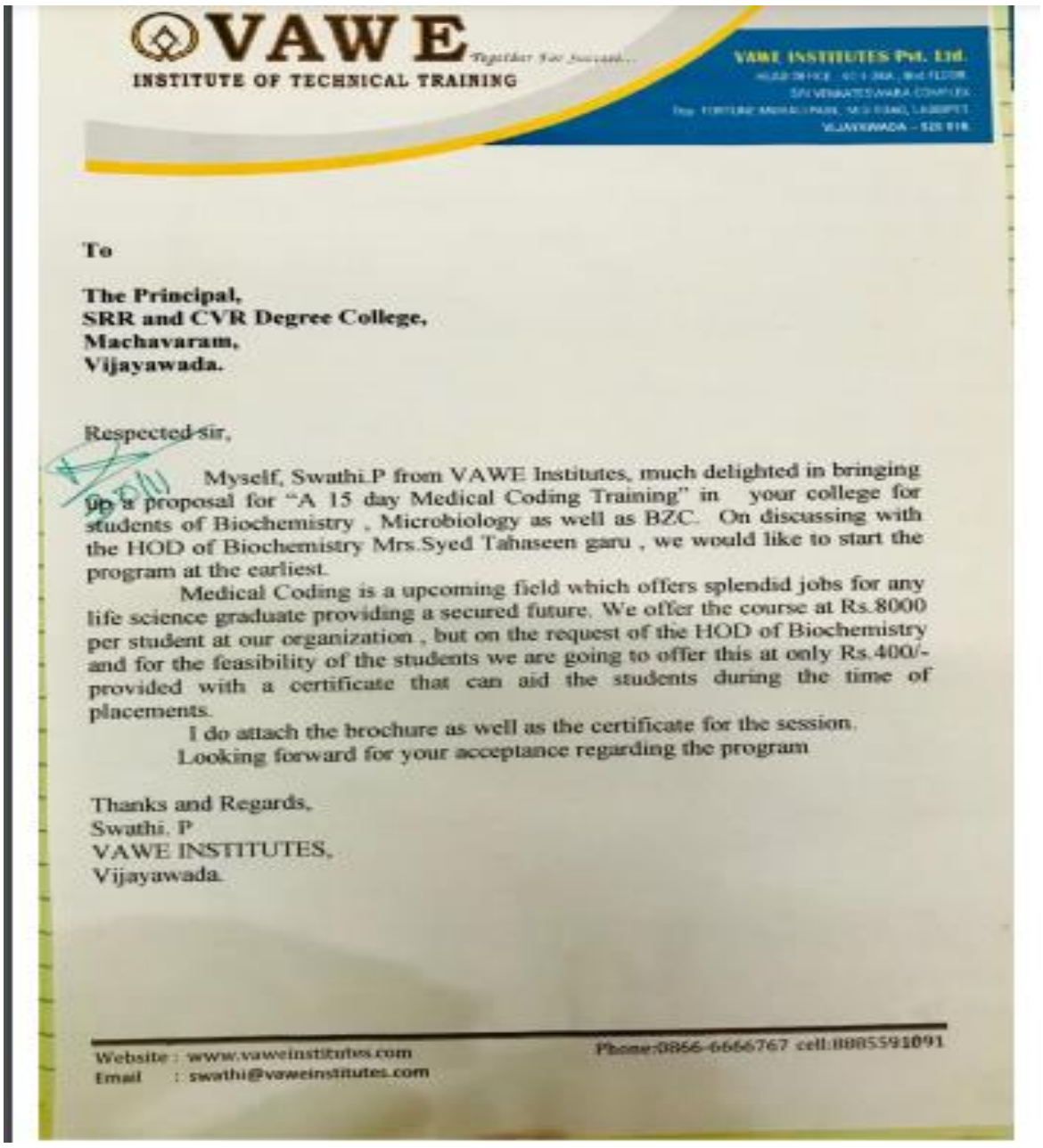
Reported by Syed Vaziha Tahaseen

The department of biochemistry has taken the initiative to start a training program in Medical coding. Medical coding is the **process of translating crucial medical information into simple codes to document medical records and for medical billing**. This standard medical coding system allows a more seamless transfer of medical records and more efficient analysis to track patients' health records. Many of the students, who are very interested in being selected in campus selections and getting immediate placements, need to be acquainted with the company's skills as Vijayawada city has many multispeciality hospitals and a massive need for medical translators. In this view, the department of biochemistry approached the **VAWE institute of technical** training to impart skills to students studying life sciences. Mrs. P.Swathi, the Trainer at the institute, came forward to provide training to our college students on the campus in addition to their regular college hours. The training program lasted for 30 days; students were introduced to all the technical knowledge required to work as a medical coder.

With the provided training, one of the students, Mr. Vishwanethtry Kanuru, of batch 2017-2019, was selected as a junior executive in medical coding at **PhyCare services, IT PARK, Mangalagiri (offer letter-page no.**

Acknowledgment: We thank our principal, Dr. Velaga Joshi, for encouraging and giving permission to organize the program.

We also show our gratitude for the VAWE institute of technical training for proving the Trainer P.Swathi for teaching the classes. We also thank our computer department, HOD, for providing the lab to organize the classes.



VAWE
INSTITUTE OF TECHNICAL TRAINING

VAWE INSTITUTES Pvt. Ltd.

To

The Principal,
SRR and CVR Degree College,
Machavaram,
Vijayawada.

Respected sir,

Myself, Swathi.P from VAWE Institutes, much delighted in bringing up a proposal for "A 15 day Medical Coding Training" in your college for students of Biochemistry , Microbiology as well as BZC. On discussing with the HOD of Biochemistry Mrs.Syed Tahaseen garu , we would like to start the program at the earliest.

Medical Coding is a upcoming field which offers splendid jobs for any life science graduate providing a secured future. We offer the course at Rs.8000 per student at our organization , but on the request of the HOD of Biochemistry and for the feasibility of the students we are going to offer this at only Rs.400/- provided with a certificate that can aid the students during the time of placements.

I do attach the brochure as well as the certificate for the session.

Looking forward for your acceptance regarding the program

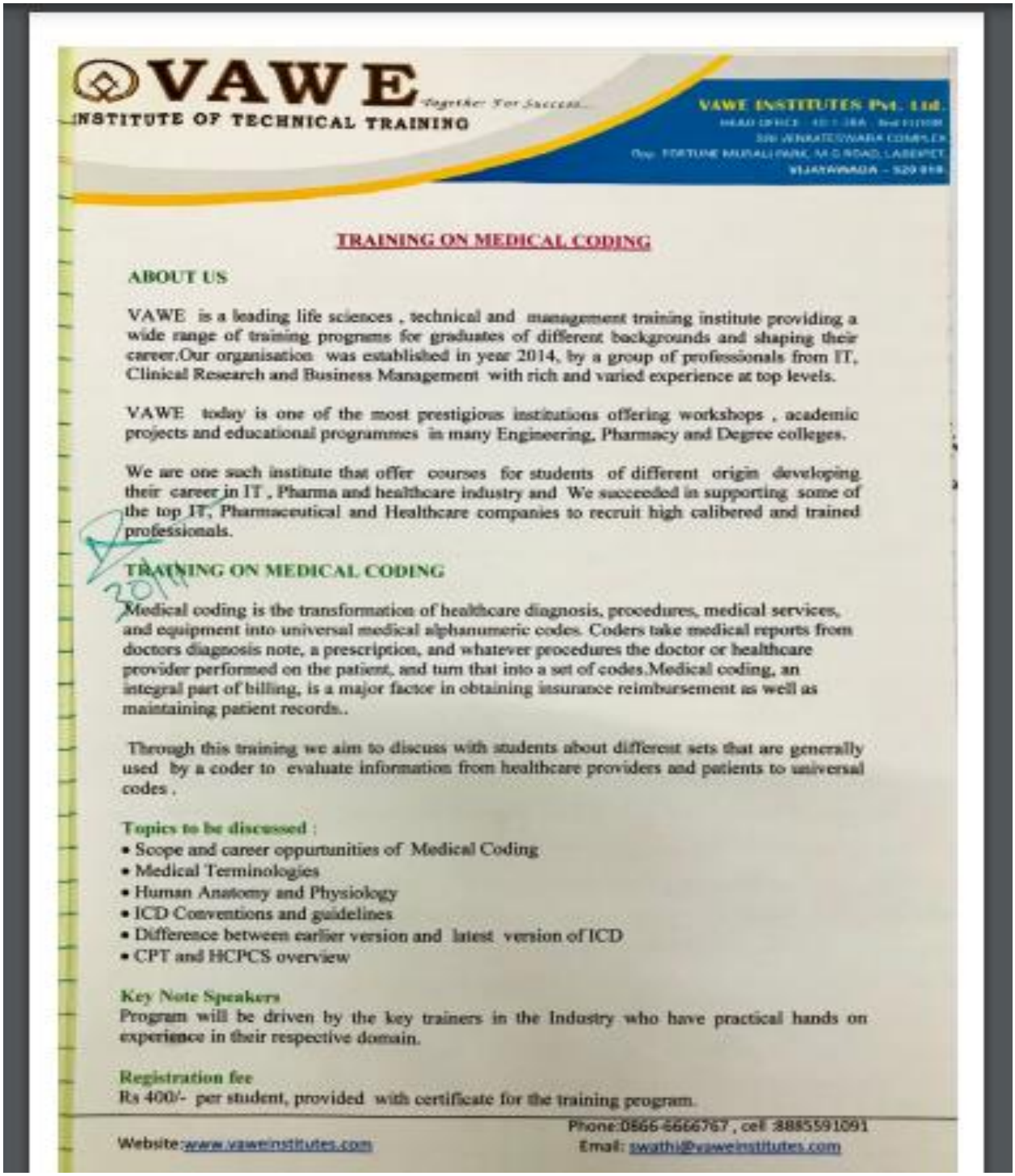
Thanks and Regards,
Swathi. P
VAWE INSTITUTES,
Vijayawada.

Website : www.vaweinstitutes.com
Email : swathi@vaweinstitutes.com
Phone:0866-6666767 cell:8885591091

Letter received from Trainer to the principal for organizing the classes in our college campus for 15 days initially, but the course was extended for another 15 days with

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry



VAWE
Together For Success...
INSTITUTE OF TECHNICAL TRAINING

VAWE INSTITUTES Pvt. Ltd.
HEAD OFFICE : 40-1-38A, 2nd FLOOR
320 VENKATESWARA COMPLEX
Opp: FORTUNE MURALI PARK, M G ROAD, LABBIPET
VIJAYAWADA – 520 010

TRAINING ON MEDICAL CODING

ABOUT US

VAWE is a leading life sciences , technical and management training institute providing a wide range of training programs for graduates of different backgrounds and shaping their career.Our organisation was established in year 2014, by a group of professionals from IT, Clinical Research and Business Management with rich and varied experience at top levels.

VAWE today is one of the most prestigious institutions offering workshops , academic projects and educational programmes in many Engineering, Pharmacy and Degree colleges.

We are one such institute that offer courses for students of different origin developing their career in IT , Pharma and healthcare industry and We succeeded in supporting some of the top IT, Pharmaceutical and Healthcare companies to recruit high calibered and trained professionals.

TRAINING ON MEDICAL CODING

Medical coding is the transformation of healthcare diagnosis, procedures, medical services, and equipment into universal medical alphanumeric codes. Coders take medical reports from doctors diagnosis note, a prescription, and whatever procedures the doctor or healthcare provider performed on the patient, and turn that into a set of codes.Medical coding, an integral part of billing, is a major factor in obtaining insurance reimbursement as well as maintaining patient records..

Through this training we aim to discuss with students about different sets that are generally used by a coder to evaluate information from healthcare providers and patients to universal codes .

Topics to be discussed :

- Scope and career oppurtunities of Medical Coding
- Medical Terminologies
- Human Anatomy and Physiology
- ICD Conventions and guidelines
- Difference between earlier version and latest version of ICD
- CPT and HCPCS overview

Key Note Speakers

Program will be driven by the key trainers in the Industry who have practical hands on experience in their respective domain.

Registration fee

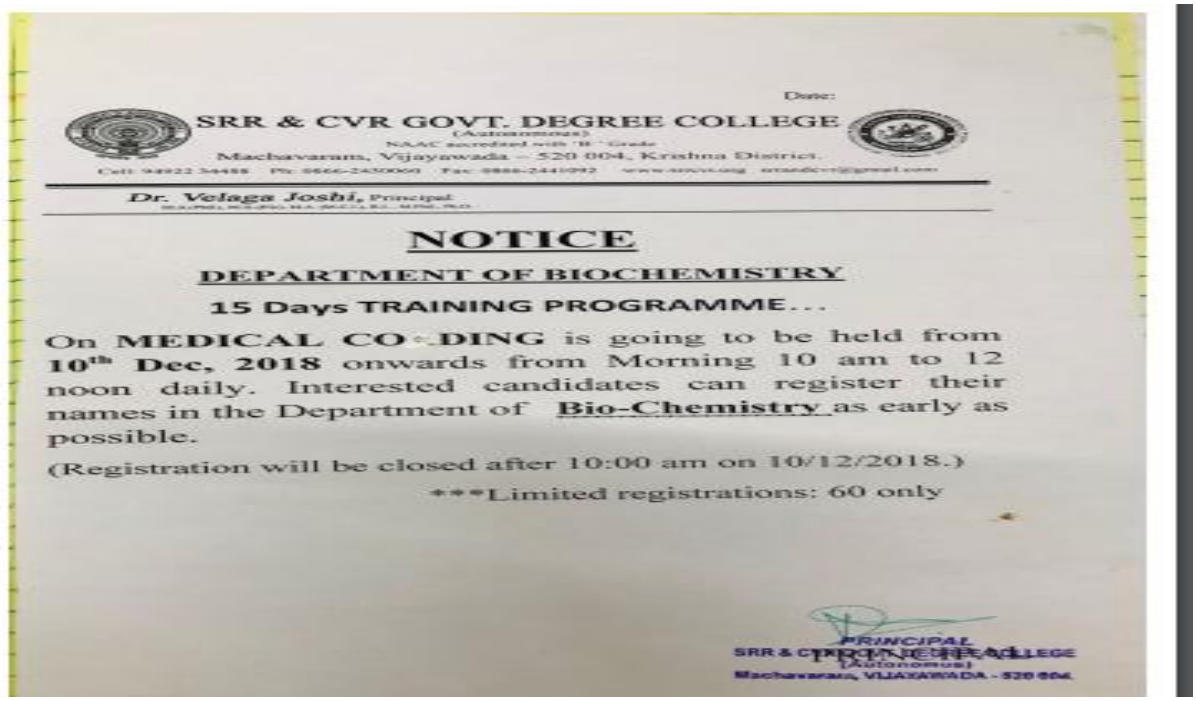
Rs 400/- per student, provided with certificate for the training program.

Website:www.vaweinstitutes.com

Phone:0866-6666767 , cell :8885591091
Email: swathi@vaweinstitutes.com

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry



Date:

SRR & CVR GOVT. DEGREE COLLEGE
(Autonomous)
NAAC accredited with 'B' Grade
Machavaram, Vijayawada – 520 004, Krishna District.
Cell: 94922 34488 Ph: 0866-2430069 Fax: 0866-2441092 www.srrcvr.org srrandcvr@gmail.com

Dr. Velaga Joshi, Principal

NOTICE

DEPARTMENT OF BIOCHEMISTRY

15 Days TRAINING PROGRAMME...

On **MEDICAL CO DING** is going to be held from **10th Dec, 2018** onwards from Morning 10 am to 12 noon daily. Interested candidates can register their names in the Department of **Bio-Chemistry** as early as possible.

(Registration will be closed after 10:00 am on 10/12/2018.)

***Limited registrations: 60 only

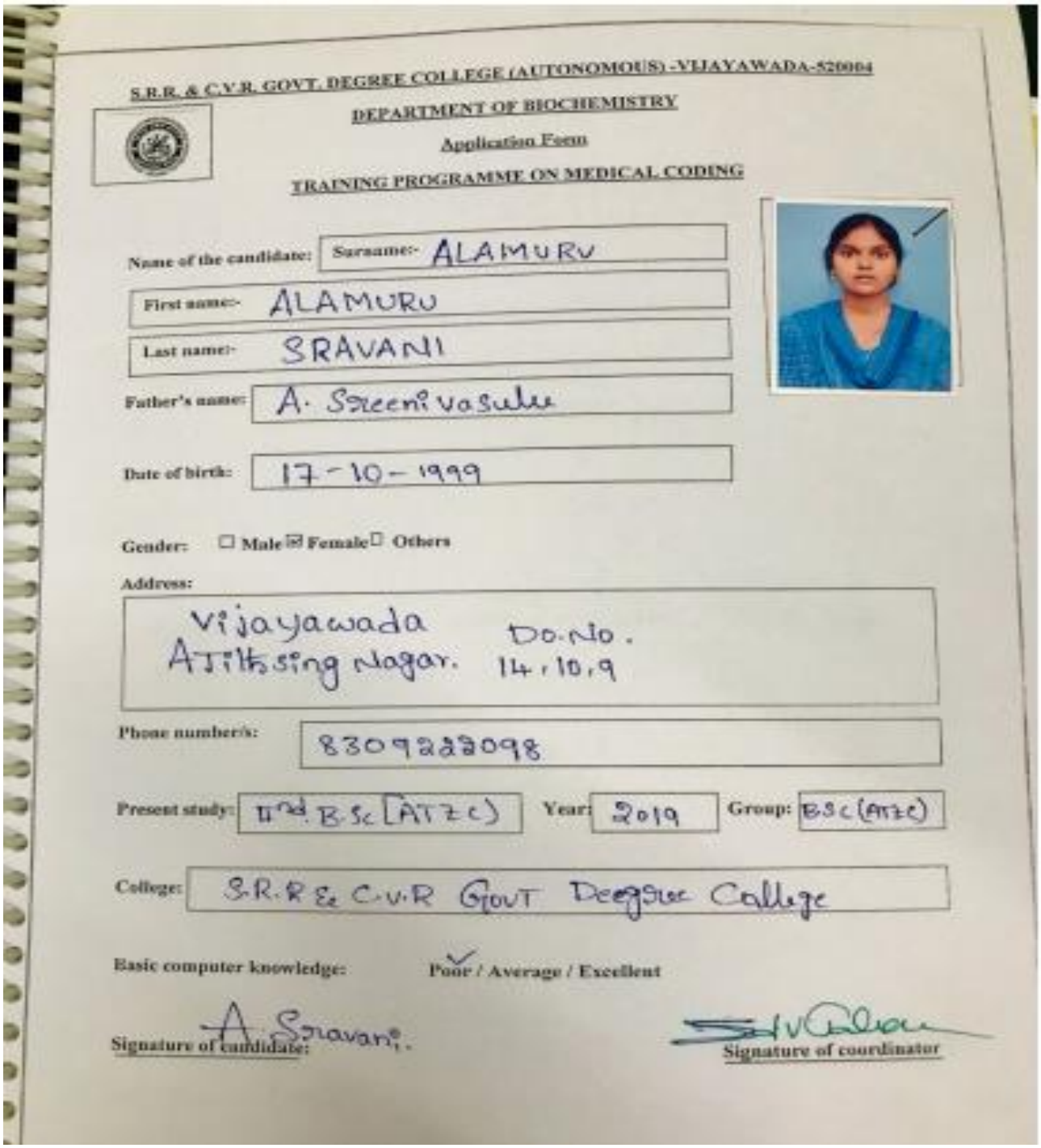
PRINCIPAL
SRR & CVR GOVT. DEGREE COLLEGE
(Autonomous)
Machavaram, VIJAYAWADA - 520 004.

Madam P.Swathi took the medical coding classes at the computer lab at our colleg

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry

Our college principal Dr. Velaga Joshi awarded certificates to the students' for successfully completing the training program, appreciated the entire team for showing their efforts.



S.R.R. & C.V.R. GOVT. DEGREE COLLEGE (AUTONOMOUS) -VIJAYAWADA-520004

DEPARTMENT OF BIOCHEMISTRY

Application Form

TRAINING PROGRAMME ON MEDICAL CODING

Name of the candidate: Surname:- ALAMURU

First name:- ALAMURU

Last name:- SRAVANI

Father's name: A. Sreenivasulu

Date of birth: 17-10-1999

Gender: ☐ Male ☑ Female ☐ Others

Address:

Vijayawada
Ajithsing Nagar.
D.No. 14,10,9

Phone number/s: 8309222098

Present study: IInd B.Sc (AHZC) Year: 2019 Group: BSc (AHZC)

College: S.R.R & C.V.R Govt Degree College

Basic computer knowledge: Poor / Average / Excellent

Signature of candidate: A. Sravani.

Signature of coordinator

Application form to be filled by the student at the time of admission in this program. **Total 47 life sciences students from the program MBC, BZC** were enrolled.

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry

SRR & CVR GOVT. DEGREE COLLEGE

(Autonomous)

Machavaram, Vijayawada, Krishna Dist - 520 004

DEPARTMENT OF BIO-CHEMISTRY

in Collaboration with

VAWE GLOBAL TECHNOLOGIES PVT. LTD.

CERTIFICATE

This is to certify that Mr. / Ms. DUSSA SAI SREE

successfully completed the Certificate Course entitled

"MEDICAL CODING" with satisfactory attendance and performance during the period

from 10-12-2018 to 10-01-2019.

Mrs. Syed Vaziha Tahaseen
Course Co-ordinator

Mrs. P. Swathi
Life Science Sector Manager

Dr. VELAGA JOSHI
PRINCIPAL

Model copy of certificate awarded for the students who have completed their training.

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

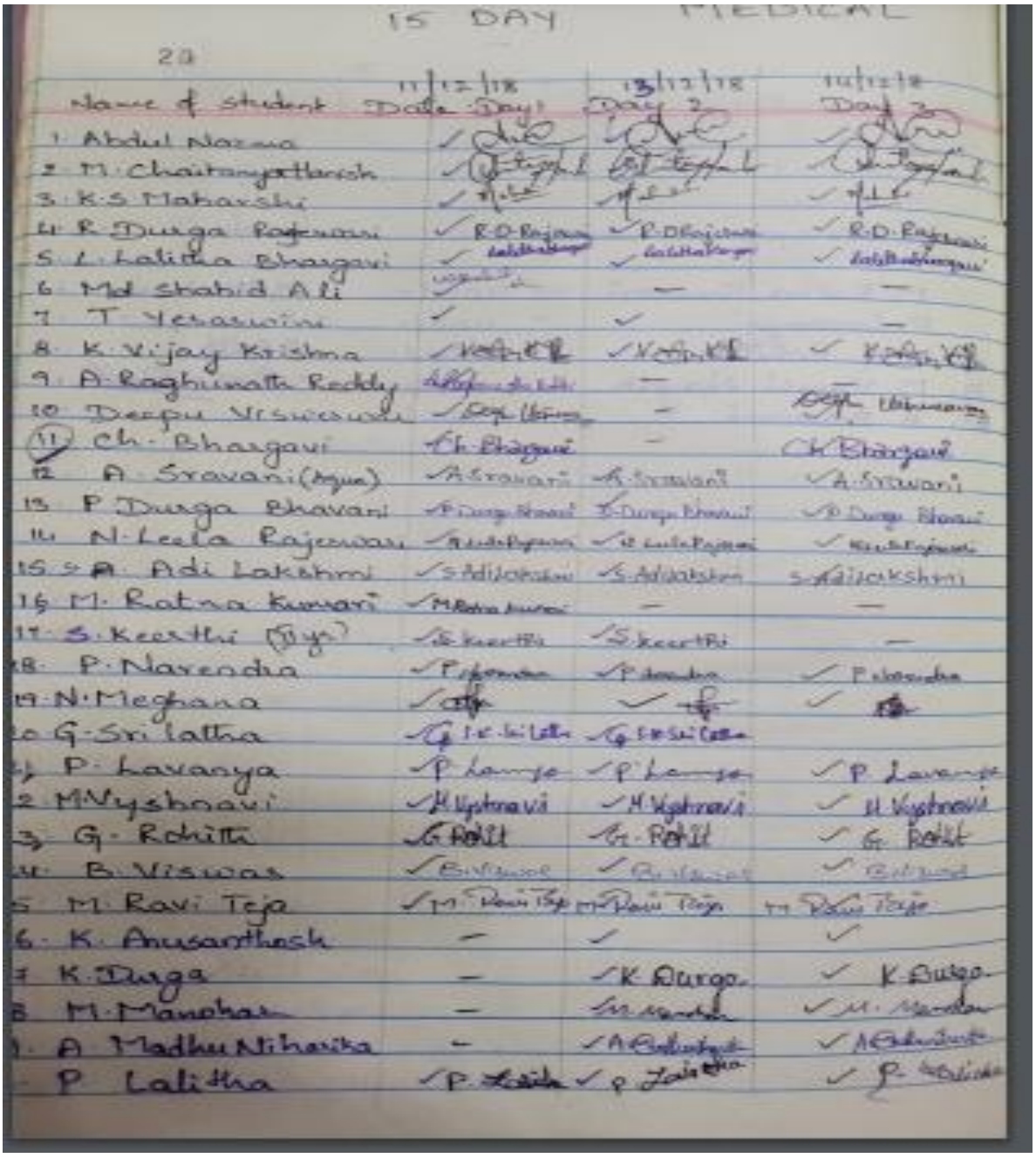
Department of Biochemistry

సర్టిఫికేషన్ కోర్సులతో ఉద్యోగ అవకాశాలు

- సుబ్బరం

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry



15 DAY MEDICAL

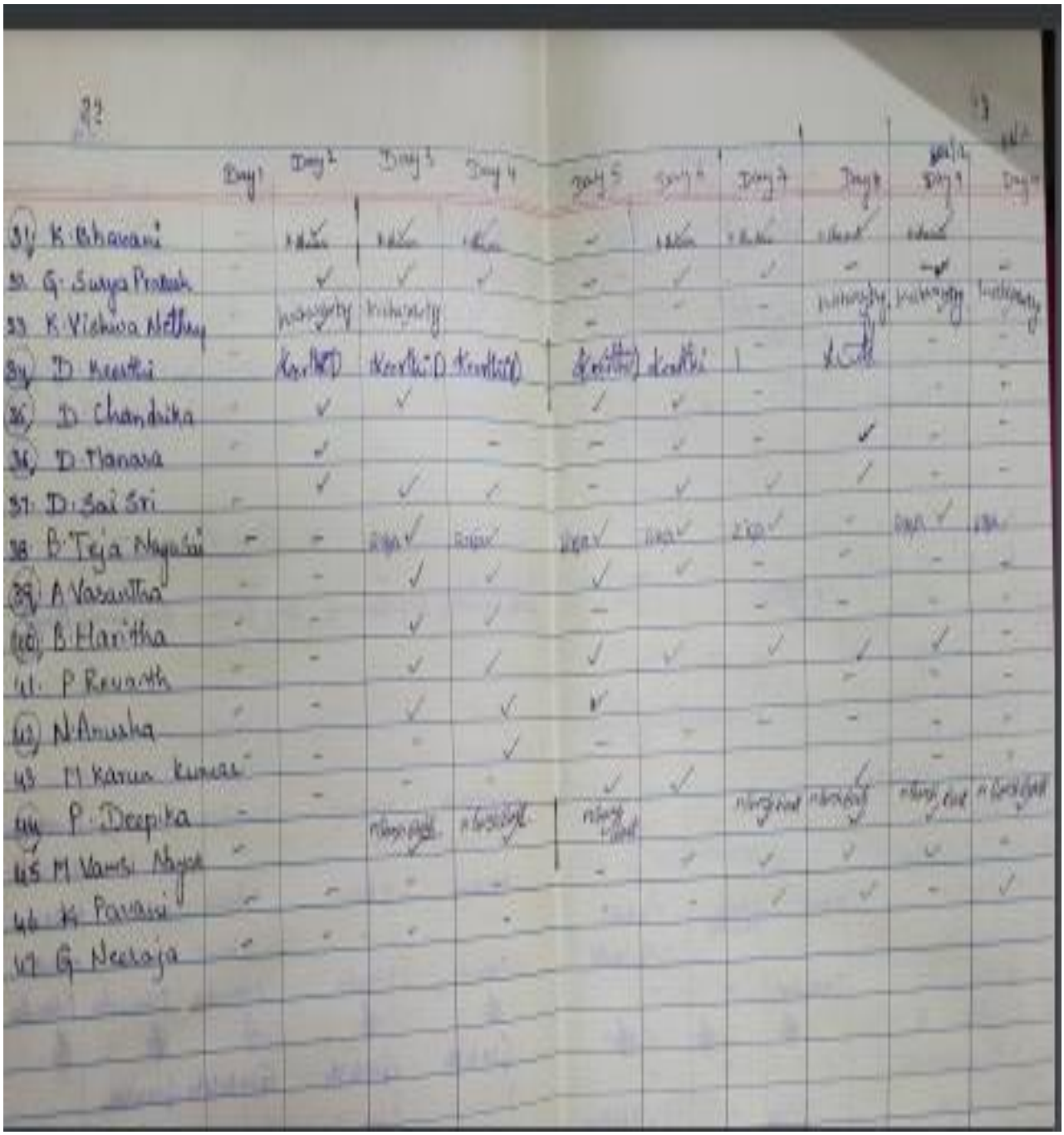
23

Name of student	11/12/18 Date Day 1	13/12/18 Day 2	14/12/18 Day 3
1 Abdul Nazeer	✓	✓	✓
2 M Chaitanyatharish	✓	✓	✓
3 K S Maharshi	✓	✓	✓
4 R Durga Rajeswari	✓ R.D.Rajeswari	✓ R.D.Rajeswari	✓ R.D.Rajeswari
5 L Lalitha Bhargavi	✓	✓	✓
6 Md Shahid Ali	✓	—	—
7 T Yesaswini	✓	✓	—
8 K Vijay Krishna	✓	✓	✓
9 A Raghunatha Reddy	—	—	—
10 Deepu Viswanath	✓	—	✓
11 Ch Bhargavi	✓ Ch Bhargavi	—	✓ Ch Bhargavi
12 A Sravani (Agua)	✓ A Sravani	✓ A Sravani	✓ A Sravani
13 P Durga Bhavani	✓ P Durga Bhavani	✓ P Durga Bhavani	✓ P Durga Bhavani
14 N Leela Rajeswari	✓ N Leela Rajeswari	✓ N Leela Rajeswari	✓ N Leela Rajeswari
15 A Adi Lakshmi	✓ S Adilakshmi	✓ S Adilakshmi	✓ S Adilakshmi
16 M Ratna Kumari	✓ M Ratna Kumari	—	—
17 S Keerthi (Jiya)	✓ S Keerthi	✓ S Keerthi	—
18 P Narendra	✓ P Narendra	✓ P Narendra	✓ P Narendra
19 N Meghana	✓	✓	✓
20 G Sri latha	✓ G Sri Latha	✓ G Sri Latha	
21 P Lavanya	✓ P Lavanya	✓ P Lavanya	✓ P Lavanya
22 M Vyshnavi	✓ M Vyshnavi	✓ M Vyshnavi	✓ M Vyshnavi
23 G Rohith	✓ G Rohith	✓ G Rohith	✓ G Rohith
24 B Viswas	✓ B Viswas	✓ B Viswas	✓ B Viswas
25 M Ravi Teja	✓ M Ravi Teja	✓ M Ravi Teja	✓ M Ravi Teja
26 K Anusanthosh	—	✓	✓
27 K Durga	—	✓ K Durga	✓ K Durga
28 M Manohar	—	✓ M Manohar	✓ M Manohar
29 A Madhu Niharika	—	✓ A Madhu Niharika	✓ A Madhu Niharika
30 P Lalitha	✓ P Lalitha	✓ P Lalitha	✓ P Lalitha

Enrolled students list

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

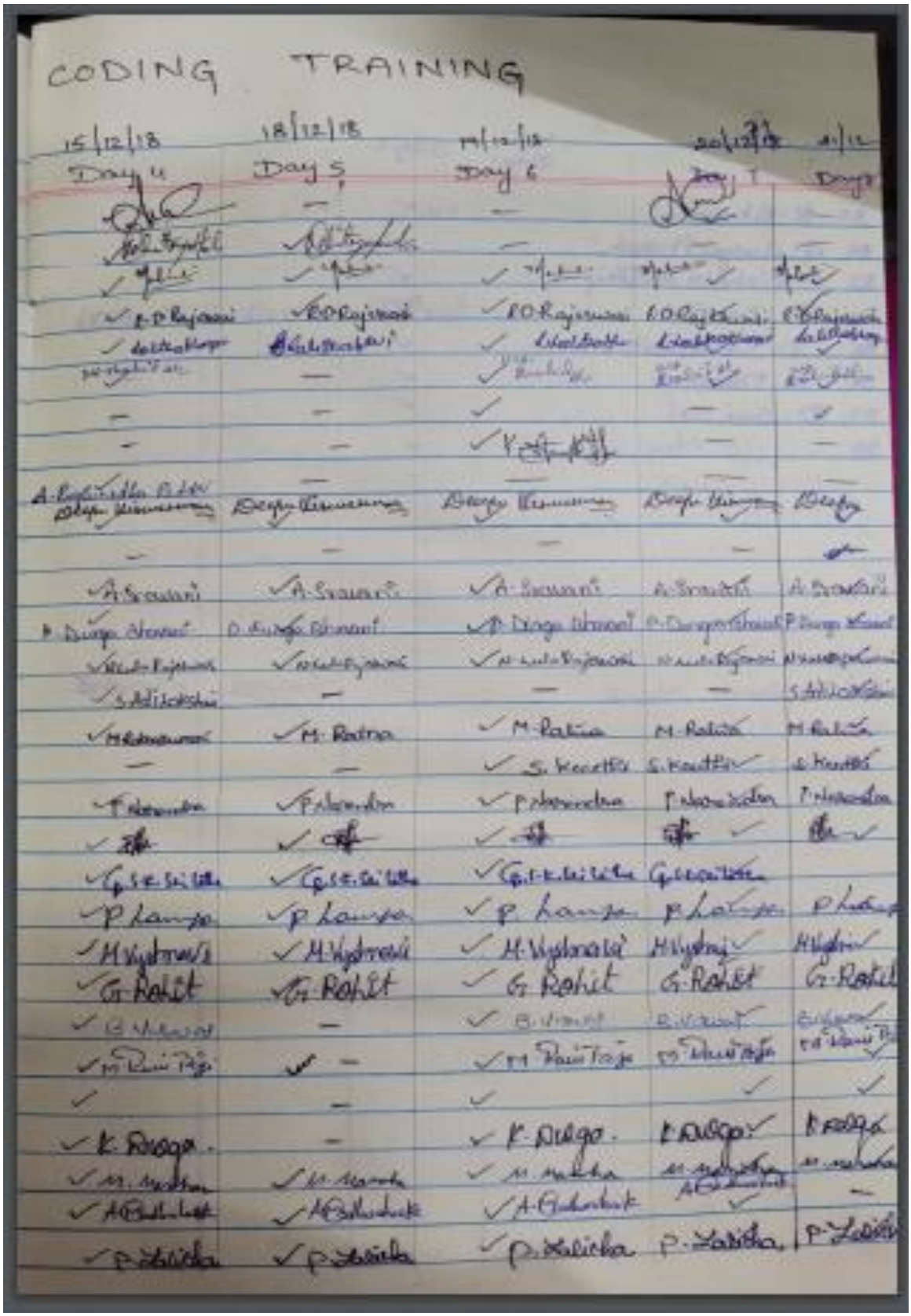
Department of Biochemistry



Student attendance

S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry



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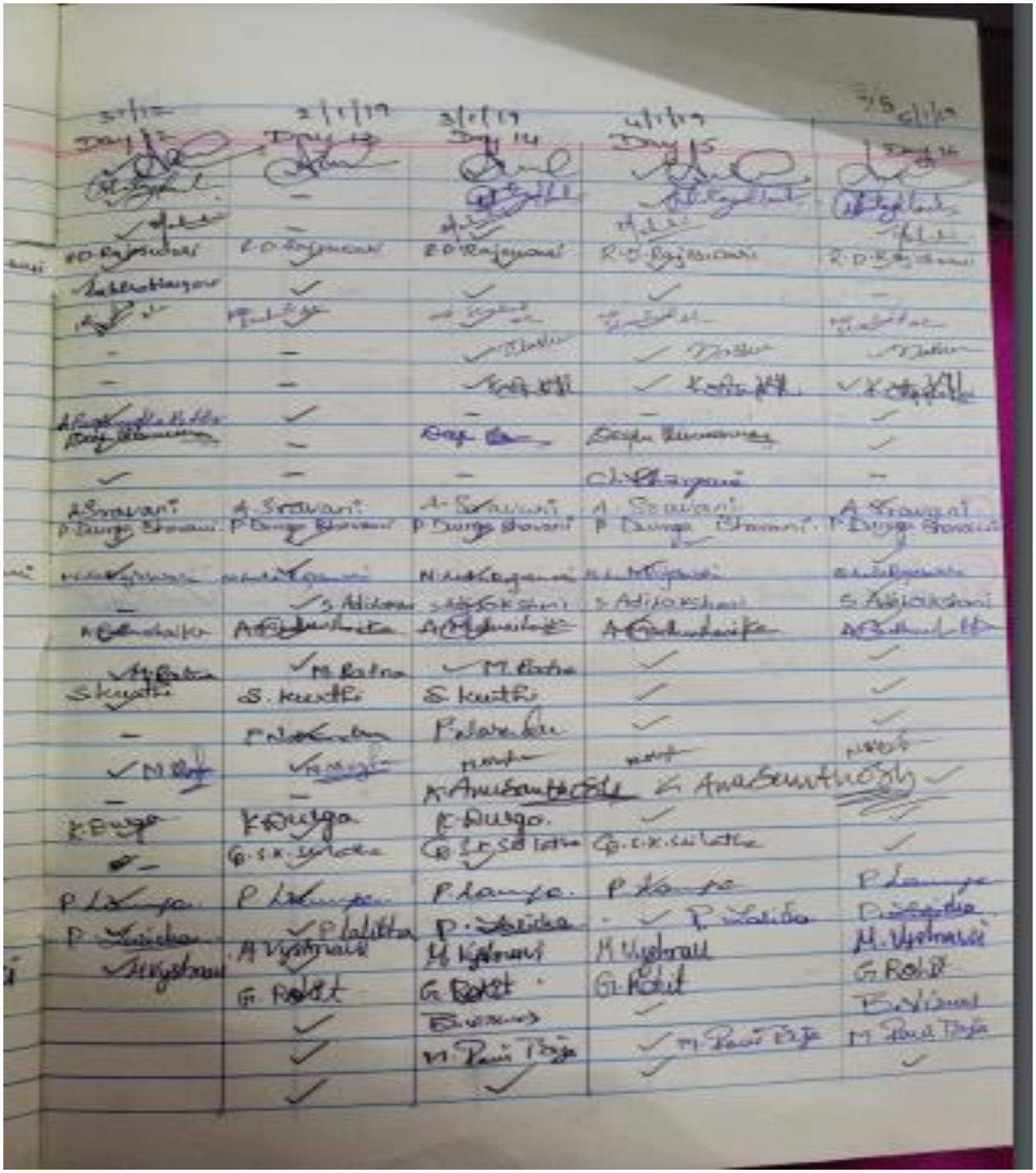
Department of Biochemistry



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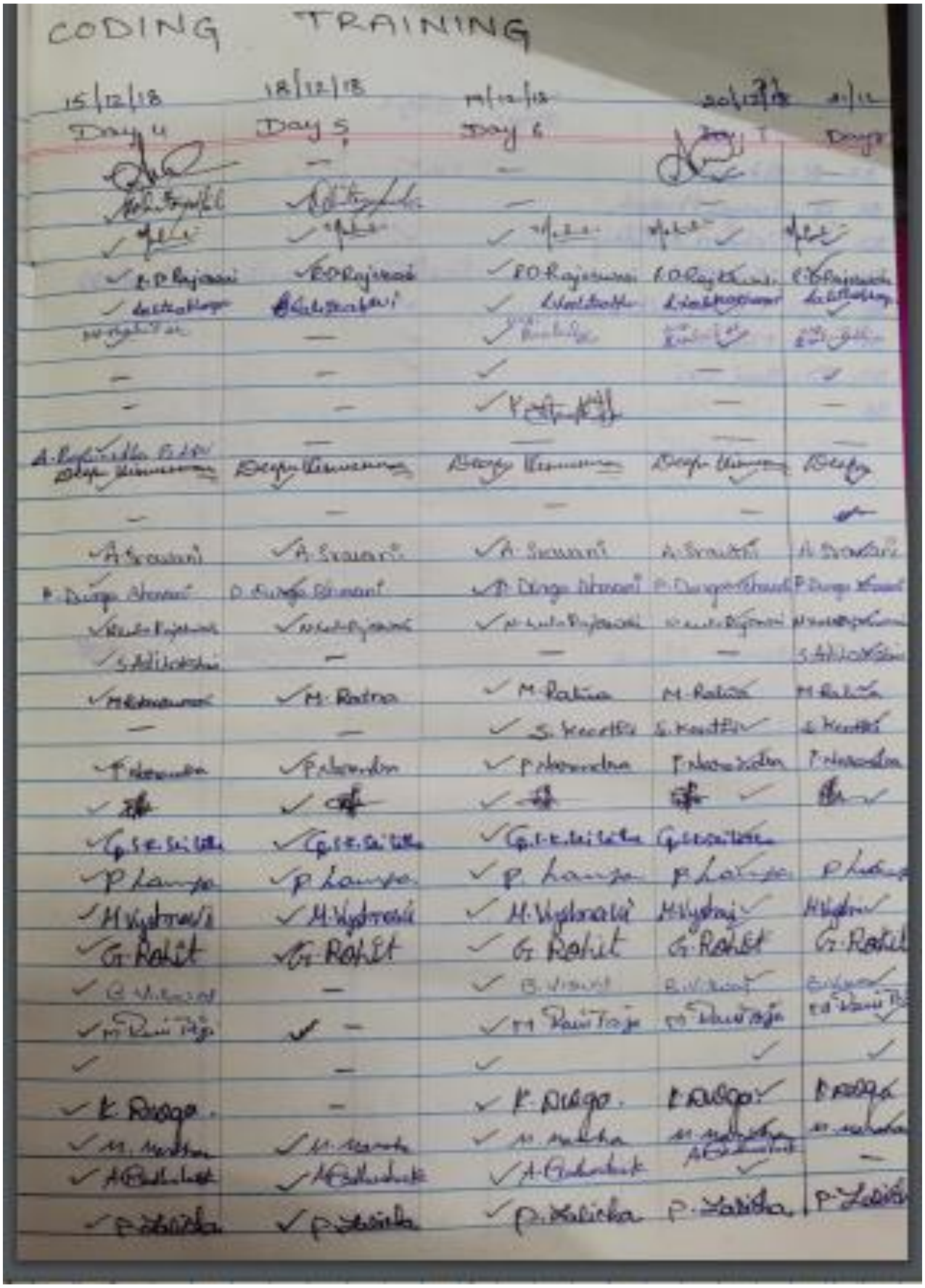

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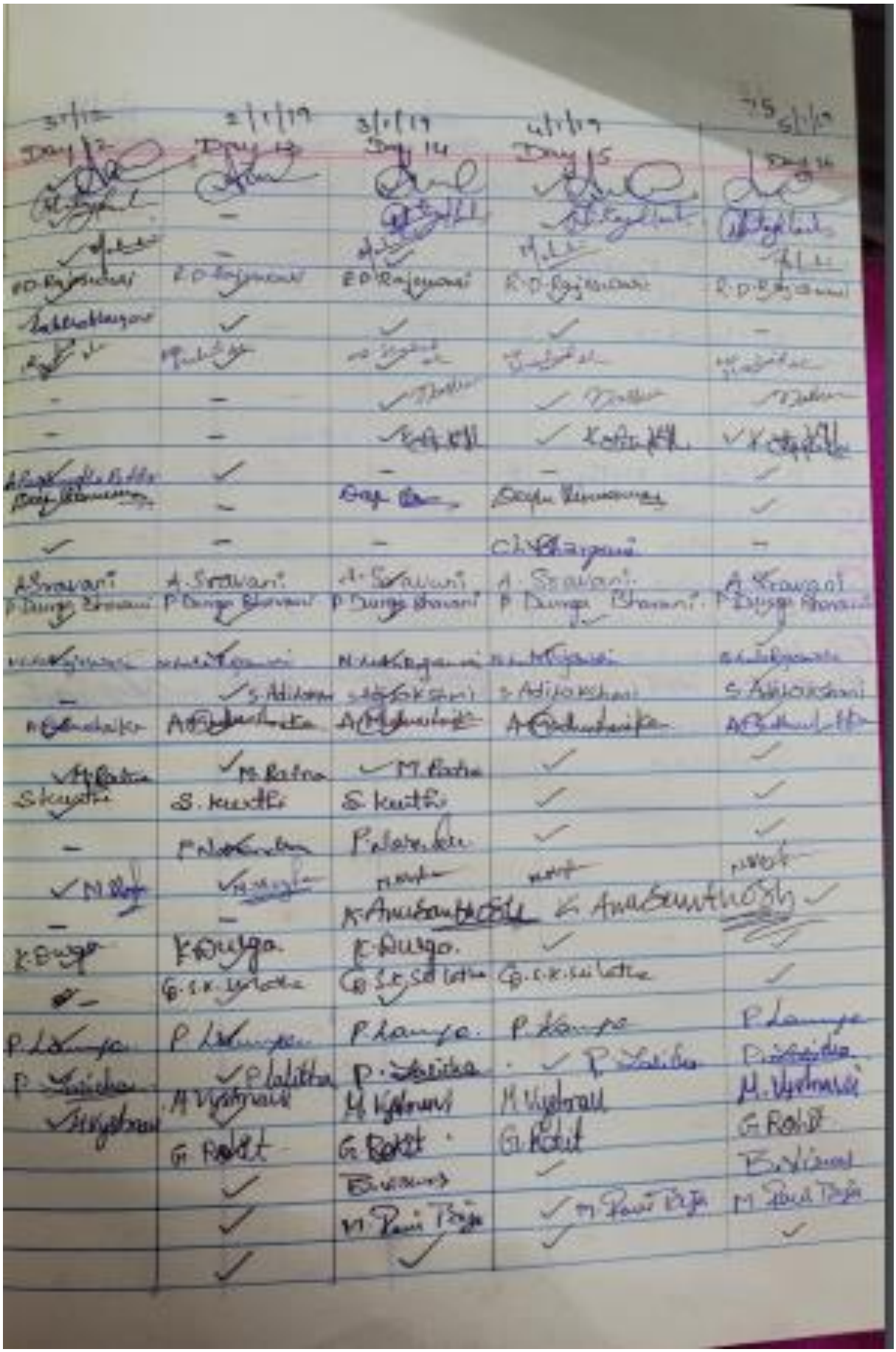

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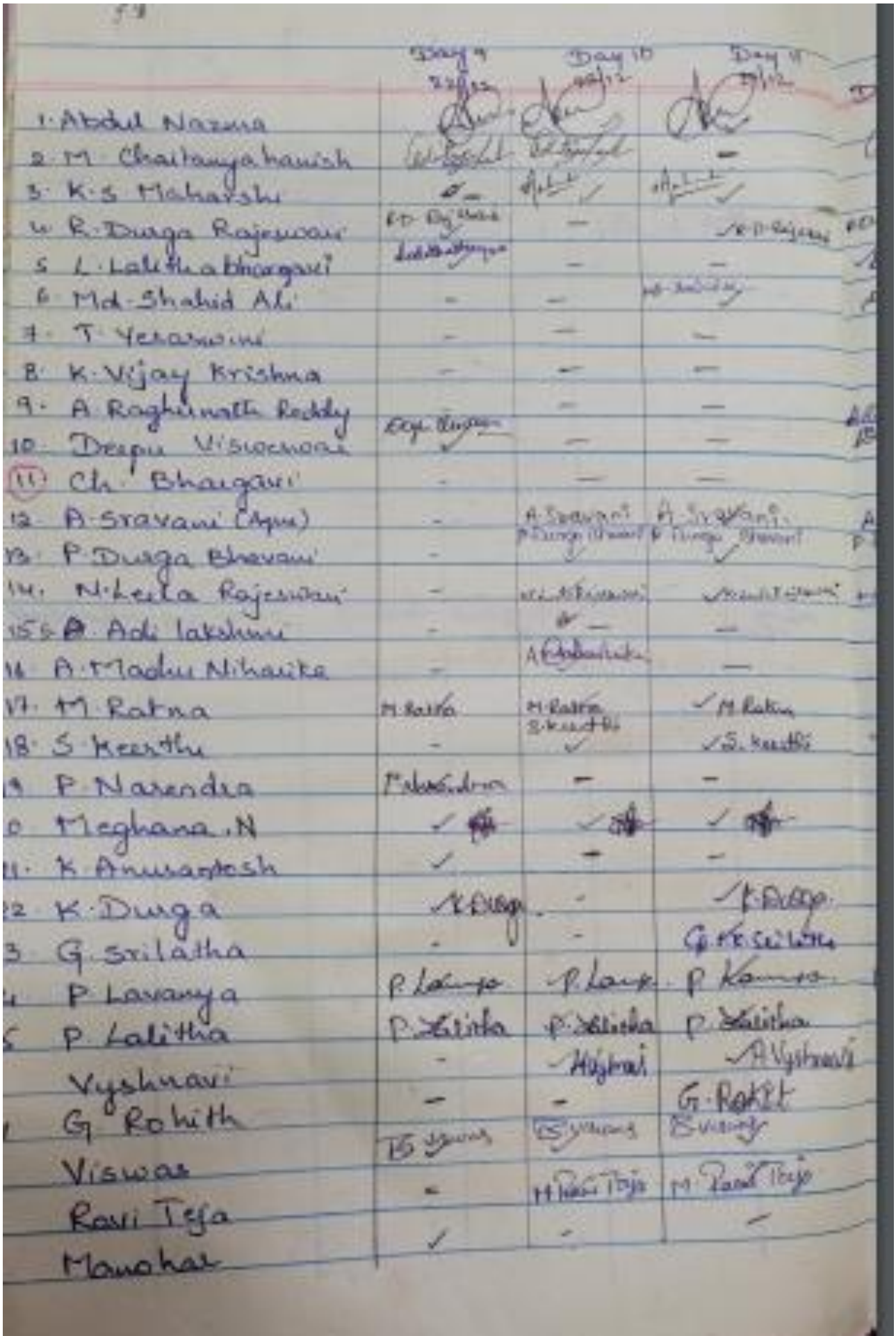

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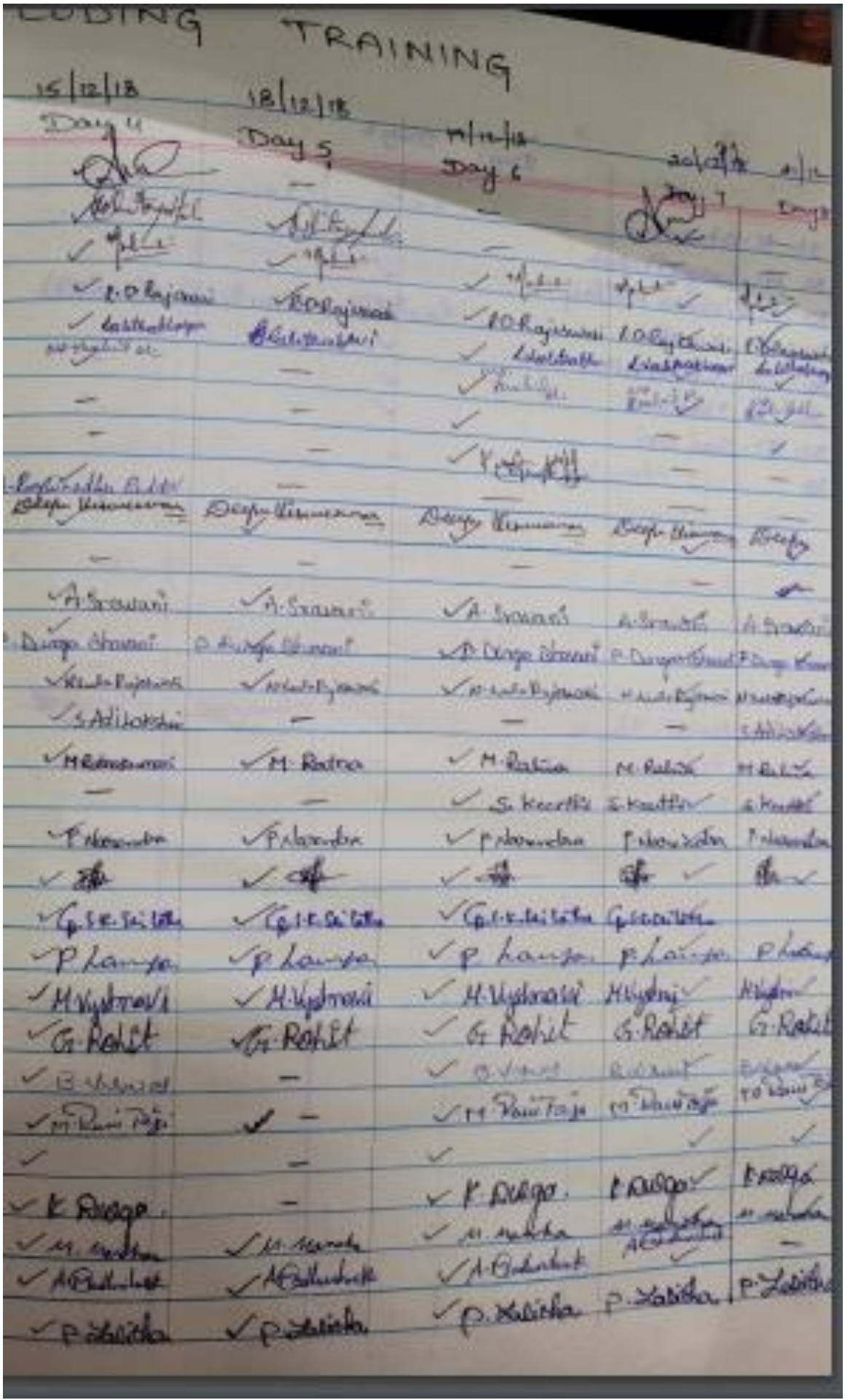

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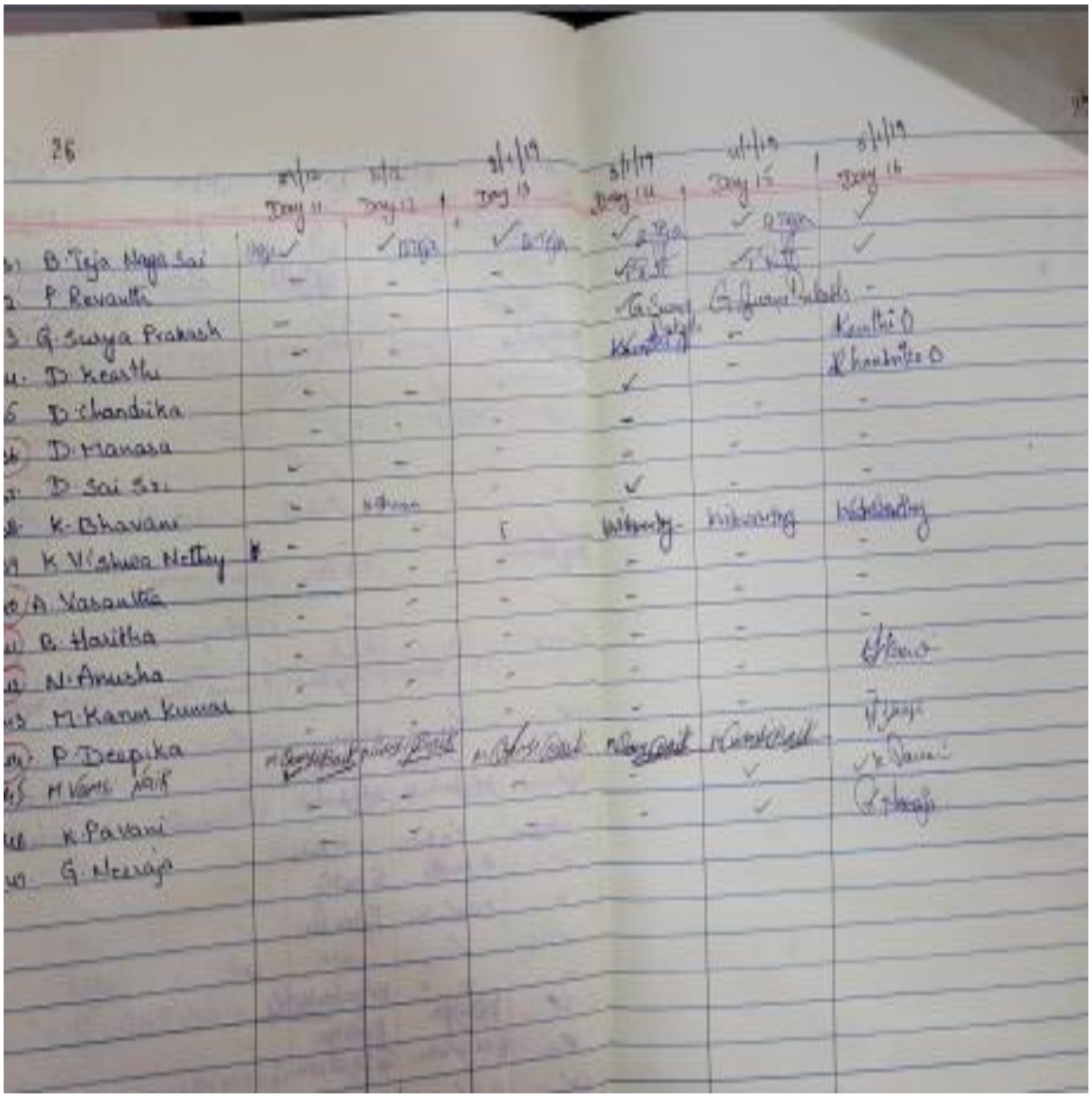
Department of Biochemistry



S.R.R.&C.V.R. GOVT.DEGREE COLLEGE(A)

Department of Biochemistry

Students successfully completed the

Medical coding program

PhyCare Services
(An ISO 9001:2008 & ISO 27001:2005 Company)

PhyCare Services India Pvt. Ltd.,
Plot No.9, Survey No.49,
IT Park, Mangalagiri,
Guntur District – 522 503
Andhra Pradesh, INDIA
Tel : +91 9394411007
www.phycaresolutions.com

Letter of Appointment

Date: 19 January, 2021
Ref: Appt./MC/HR/01/2021

Dear **Mr. Vishwanethry Kanuru,**

We are happy to offer you the letter of appointment with following position in our Organization under the terms and conditions indicated below:

Position: Junior - Executive

Department: Medical Coding - RCM

Date of Joining: 19 January, 2021

Your consolidated salary and other benefits (CTC) have been fixed at **INR. 10,000.00 (Rupees Ten Thousand Only)** per month payable on the 5th of the subsequent month. The payment of salary and other benefits will be subject to the deduction of Income Tax in accordance with the provisions of the Income Tax Act, 1961, and provisions of other applicable statutes, as at the time of payment.

All statutory deductions viz., PF, Professional Tax, IT, if applicable, will be done as per the prevailing rules of the Government and employers' contribution, where applicable, will be paid to the concerned department to the credit of your account. Gratuity and other benefits will be as per law. And annual performance incentives will be as per Company policy.

Income Tax Liability

The Income Tax Liability with regards to your salary and perks will be your liability, and will be governed by the tax laws of the country as applicable from time to time including TDS. You will have to work out your tax planning with us and advise us accordingly for IT TDS deduction failing which, the company will make the necessary calculations based on assumptions and deduct the TDS as shown above, in which case you will have to apply for refund of excess tax paid, if any, with IT Dept., on your own.

The cost to the company (CTC) of your employment works out to INR. 10,000.00.

Probationary Period: (IF mentioned as NIL in the period of probation, this section is not applicable to you).

You will be on probation for a period of **Six Months** from the date of joining. Upon satisfactory completion of this period & after the background verification which should be positive, you may be confirmed in the regular cadre of the Company.

During the period of probation, in the event of your resignation/leaving the company you will be required to give 60 days' notice to ensure smooth transition/takeover of duties without loss to the Company. In cases where adequate notice is not given, salary for the period of shortfall will be deducted while carrying out the full and final settlement. Also, during the period of probation, the Company may terminate the services of an individual without assigning any reasons, but with a minimum of one-weeks' notice or salary in lieu thereof. However, the management reserves the right to waive or reduce the notice period required to be given by the employee based on special circumstances of each case. Subsequently, on confirmation, you will have to give 60 working days notice. In the event that requisite period of notice is not being given by the employee, they will be liable to compensate proportionately to the extent of salary and allowances due for the period of shortfall in notice period or as mentioned in relieving point.

Professional Ethics

You are required to deal with the Company's money, material and documents with utmost honesty and professional ethics. If you are found guilty, at any point of time of moral turpitude or of dishonesty in dealing with the Company's money or material or documents or of theft or of misappropriation, regardless of the value involved your services would be terminated with immediate effect, not withstanding other terms and conditions mentioned in the appointment letter.

Performance Appraisal

Your next review of salary will be done on satisfactory completion of one-year service with us, subject to your appointment being confirmed as permanent, if you have been appointed on a probationary basis. Your performance appraisal will be conducted as per prevailing Company's policy. There shall be no automatic increments expect performance based incentives that may be given as per the Company policy for rewarding the performance of individuals after one year. The performance will

PhyCARE-HRD/2021-APL-4.0 Page 1 of 6 Confidential

in turn depend on the business prosperity appraisal of the whole business of the Company as conducted by the management. Your promotion to other positions or opportunities depends on your performance as mentioned above. And at point if your performance is not appreciated or not according to the standards then it can also be a negative appraisal or even no appraisal at all, until your performance is at par with others or as per the standards.

General

- You agree to devote your full time and ability to the Company and shall not engage yourself in other employment, business or vocation, whether part time or full time and whether with or without necessary benefits, without the prior written consent of the Company.
- You agree to comply with and abide by the policies, procedures, guidelines, code of conduct, standing orders and other rules and regulations of the Company that may currently be in-force or that may be issued or communicated to you from time to time, through the Employees' Manual, Circulars, Notices or otherwise.
- Any payment, not due to you, but made to you by the Company as a result of clerical/supervisory lapse, though may have recurred, will not entitle you to such payments as a matter of right. Management has the right not only to stop such payments, once detected, but will also recover such payments made earlier from amounts payable to you subsequently.

Offer letter received by Mr. Vishwanethtry Kanuru, of batch 2017-2019, was selected as a junior executive in medical coding at PhyCare services, IT PARK, Mangalagiri.

SRR & CVR GOVERNMENT DEGREE COLLEGE (A)
VIJAYAWADA

DRC conducted the Workshop on SVEEP 2019 March.in collaboration with District revenue administration

The SRR & CVR Degree College (A), Vijayawada, the District Resource Center, Krishna along with the Krishna, District revenue administration conducted the Systematic Voter Education Enrollment Program in the month of March 2019. The Principal of SRR & CVR Degree College (A), Vijayawada Dr.Velaga Joshi aired his views on the significance of vote in democracy. Activities under the Systematic Voters' Education and Electoral Participation (SVEEP) Programme are undertaken to educate the electors regarding procedures relating to registration of the name in Electoral Roll, correct of their existing particulars in Electoral Roll and deletion of the name of shifted and deceased family members.

Activities under the Systematic Voters' Education and Electoral Participation (SVEEP) Programme are undertaken to educate the electors regarding procedures relating to registration of the name in Electoral Roll, correct of their existing particulars in Electoral Roll and deletion of the name of shifted and deceased family members. SVEEP activities are also meant to inform about various online, and offline facilities available to voters regarding ethical voting like how to cast vote, how to help the election machinery prevent corrupt practices during elections etc. For awareness of general voters, following SVEEP activities have been initiated by the office of Chief Electoral Officer-Vijayawada.

Prayer by the students of SRR &CVR GDC(A) Vijayawada, during the SVEEP programme. Activities under the Systematic Voters' Education and Electoral Participation (SVEEP) Programme are undertaken to educate the electors regarding procedures relating to registration of the name in Electoral Roll, correct of their existing particulars in Electoral Roll and deletion of the name of shifted and deceased family members.

SRR& CVR GDC(A), student Miss V.Priyanka of IBSC(MECS) spoke on the importance of casting vote, voter registration and the importance of voting by the citizens.

Systematic Voters' Education and Electoral Participation program, better known as SVEEP, is the flagship program of the Election Commission of India for voter education, spreading voter awareness and promoting voter literacy in India. organisation registered under relevant Central or State Government Act can take part in the Institutional category. ➢ The participant shall give a brief description of the entry along with the name, address, and phone number. ➢ The participant shall email the entries along with the details to voter- contest@eci.gov.in.

The workshop was attended by District Collector, Krishna Sri Mohd.Imtiaz IAS and spoke to the SRR& CVR College students about the importance of vote and voter registration. Since 2009, Election has been working towards preparing electors and equipping them with basic knowledge related to the electoral process. SVEEP's primary goal is to build a truly participative democracy in India by encouraging all eligible citizens to vote and make an informed decision during the elections.

The rally took place in Vijayawada by the students of SRR & CVR GDC(A),Vijayawada. Specifically, this citizen-voter education module aims to: -acquaint the electorate with the basic concepts of democracy and the role of elections in democracy and governance; -stress the importance of one's vote; -encourage voters to participate in the whole electoral and governance process; and -eventually effect ...

By providing unbiased, non-partisan information, voters are able to connect directly with the facts so they can: Register to vote. Learn about the candidates and issues on their ballot. The law does not require citizens to vote, but voting is a very important part of any democracy. By voting, citizens are participating in the democratic process. Citizens vote for leaders to represent them and their ideas, and the leaders support the citizens' interests.

Rangavalli competitions were conducted in all colleges in the district and prizes were also distributed to the students. In order to encourage more young voters to take part in the political process, Government of India has decided to celebrate January 25 every year as "National Voters' Day". It has been started from 25 January 2011 to mark Commission's foundation day.

The students participated in Rangavalli Competitions in the college premises. Rangavalli reflects the theme of SVEEP means Systematic Voter Education and Enrollment Program. SVEEP's primary goal is to build a truly participative democracy in India by encouraging all eligible citizens to vote and make an informed decision during the elections. The programme is based on multiple general as well as targeted interventions which are designed according to the socio-economic, cultural and demographic profile of the state as well as the history of electoral participation in previous rounds of elections and learning thereof.

Essay writing competitions were conducted across the Krishna district and students enthusiastically participated from all colleges. Systematic Voters' Education and Electoral Participation program, better known as SVEEP, is the flagship program of the Election Commission of India for voter education, spreading voter awareness and promoting voter literacy in India. Since 2009, we have been working towards preparing India's electors and equipping them with basic knowledge related to the electoral process.

District level meetings were conducted at Kalakshetram, Vijayawada and all-district level heads of the departments participated. The Election Commission has declared 2019 as the 'year of the electoral roll'. The focus is on 'Purifying the electoral roll' by including more eligible voters particularly youth, women, and the Homeless' and deletion of non-existent with the due process of law. Summary Revision is done every year based on the qualifying date as of 1st January based on the Election Commission's directions and the time frame prescribed by them. There is a lot of gap between what the voters should know and what they actually

know in important areas related to Election function and management. This knowledge needs to be addressed by election Managers with a sense of urgency. Experience showed that even greater awareness does not necessarily get converted into greater Participation and the answer to this question has been found in voter education which is the most appropriate way to improve participation in a democracy in a country like India. We know that this is not a simple exercise though we have tried our level best to make this plan specific to our district and with this background; we are submitting our SVEEP Plan for Voter Registration of our District to achieve the goals and objective.

Overview - In the country like India, the legal age of casting the vote for assembly and parliamentary elections is 18 years and above. The Election Commission of India (ECI) has been consistently placing its indomitable efforts to encompass the entire citizen of India aiming that the eligible age group should be registered in the electoral list and must participate in the voting process. To a greater extent, these efforts have produced results but a lot of efforts need to be done in this direction and being done The issues like low understanding of the importance of the electoral process, thin participation of women at polling Booths, accessibility of ostracized sections of the society to polling booths and intimidated voters still pose challenges for the system. To address all such issues, the Election Commission of India has unfolded the strategy of SVEEP. When an assembly election in Andhra Pradesh is on the cards and the Parliamentary election is expected to be held in the year 2018, SVEEP adopts its vitality. SVEEP stands for Systematic Voters' Education for Electoral Participation, a process of concerted interventions to add people to the electoral process by sensitization and facilitation and ascertains increased participation of eligible age group people across all the sections and gender of society. When the electoral situation described in this report is seen, it is clear that certain polling booths have shown dismal turnout and poor participation of women voters. In such a context, the significance of SVEEP sounds relevant. SVEEP focuses on a targeted approach to bridge the gaps in enrolment.

SVEEP 2019 PROGRAMME SHEET

Calendar of SVEEP Activities

Krishna District

S. No	Date	Activity to be taken up
1	25-02-2019	• Roll-out of SVEEP Campaign • Flaging-off of the mobile awareness vehicles • Unveiling of Krishnayya Thatha
2	26-02-2019	• Slogan & Poster Making Competition- College Level
3	27-02-2019	• Human chain in all colleges • Voter Facilitation Counters in cinema halls & malls to be set up
4	28-02-2019	• Human chain in all schools • Slogan & Poster Making Competition- Inter College (Constituency Level)
5	01-03-2019	• Essay Writing & Debate Competitions in colleges • Know Your BLO Campaign kickstart
6	02-03-2019	• Drawing/ Painting Competition in all schools • Pledge to Vote Campaign starts
7	03-03-2019	• Street Plays • Slogan & Poster Making Competition- District Level
8	04-03-2019	• NCC Cadets rally
9	05-03-2019	• Essay Writing & Debate Competitions- Inter College (Constituency Level)
10	06-03-2019	• Rangoli Competitions for women
11	07-03-2019	• Technology Solutions for the voting process (Hackathon/ Competition in engineering colleges) • Essay Writing & Debate Competitions- District Level
12	08-03-2019	• Women Voters Walk • Rangoli Competitions Winners announcement
13	09-03-2019	• Campus Ambassadors announcement
14	10-03-2019	• Flash mobs by college students in malls
15	11-03-2019	• Meeting with all campus ambassadors
16	12-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
17	13-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
18	14-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
19	15-03-2019	• Human chain with PwDs
20	16-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
21	17-03-2019	• Collaborations with CSR/ Corporates to be finalized
22	18-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
23	19-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
24	20-03-2019	• Campus Connect- Interaction of eminent speakers with college students • Cultural Programmes at Ghats
25	21-03-2019	• NSS Volunteers rally
26	22-03-2019	• 2k Run
27	23-03-2019	• Street Play in a mall • Young Voters Festival (winners of all competitions to be given prizes)
28	24-03-2019	• Closing night of cultural programmes
29	25-03-2019	• SVEEP Campaign ends

Additional activities to be undertaken:

1. Voter Facilitation Counters in Cinema Halls & Malls: From 27-02-2019 to 25-03-2019 (to be set up at the entrance of the cinema hall/ mall)
2. Street Plays/ Kala Jathas: From 03-03-2019 to 25-03-2019 (will be scheduled constituency-wise)
3. Continuous engagement with all stakeholders through social media
4. Krishnayya Thatha (Campaign Mascot) will be created and used extensively to engage with the voters

S.R.R. and C.V.R. Govt. Degree College (A)
Machavarm, Vijayawada

Department of Microbiology in Association with WIPRO-GE HEALTHCARE, TATA TRUST AND GRAM TARANG

(CENTURION UNIVERSITY)

Certificate Course On

OPERATION THEATRE AND ANESTHESIA TECHNOLOGY

Course Duration: Six Months May 11th 2018 to Nov 15th 2018

Objectives:

The course is designed to meet the growing demand of experts in the fields of anaesthesia technology & operation theatre management, medical ethics, post operative care.

The course entails training of students in both theoretical and practical aspects of operating room technology. The student would be trained in various aspects of patient care, pre operative preparation, instrumentation, sterilization, operating room concepts, anesthesia, operating techniques and assistance to surgeons. On completion of the course the student would be able to perform as an operating theater nurse/ technologist in all aspects of surgical intervention in patient care.

They learn the utilization of numerous diagnostic equipments and also patient assessment abilities. Training also concentrates on the knowledge and skills of monitoring infection control policy and procedures in operation theatre.

List of Students: 22

S No	Name of the Student	Class
1	KAMABATTULA BHARGAVI	**III BSc MBC**
2	MERUGUMALA SRAVANI	
3	DEVULURI TONNY	
4	NETHALA JAYABABU	
5	MANDADAPU V VENUGOPAL	
6	MOHAMMED SOHAIL	
7	THOTA V S K MANIKANTA	
8	VALLURI LAKSHMINARAYANA	
9	GANDIKOTA NAGA PRATHYUSHA	
10	GUNTUR PRAVALLIKA	
11	GUBBALA NAVYASREE DURGA	
12	KOPPULA UDAY BHANU	
13	NEMALIKANTI SUDHAKAR	
14	ALLU INDU	
15	BOMMIDI PRASANTHI	
16	DEVADA SANTHOSI KUMARI	
17	PAPABATHINA ROJA	
18	BEZAWADA BLESSI	
19	BEJAWADA RANI PRAMILA	
20	BEVARA DIVYA	
21	RALI KALYANI	
22	BOGGAVARAPU RAJA KUMARI	

Student hands-on training in procedures


ప్రశాంత్ హాస్పిటల్

IN ASSOCIATION WITH

WIPRO-GE HEALTHCARE, TATA TRUST AND GRAM TARANG (CENTURION UNIVERSITY)

COURSE: OPERATION THEATRE AND ANESTHESIA TECHNOLOGY

CENTRE: SRR & CVR GDC (A), VIJAYAWADA

DATE OF EXAM- 27 AUGUST 2018 TIME- 10:15 AM TO 11:45 AM (90 MINS)

TOTAL MARKS- 80 PASS MARK- 50/80

INSTRUCTIONS

- EACH MODULE/SUBJECT WILL BE VALIDATED SEPARATELY. HENCE DONOT COMBINE ALL ANSWERS. USE SEPARATE PAPER FOR EACH SUBJECT WITH HEADING.
- NO MARKS FOR MISMATCHED QUESTION NUMBER TO ANSWER.
- DONOT WRITE QUESTIONS ON ANSWER SHEET. JUST MENTION QUESTION NUMBER TO ANSWER.
- NO COPIES ALLOWED.
- ANSWER ALL THE FOLLOWING QUESTIONS.

ALL THE BEST

ANATOMY AND PHYSIOLOGY – 25 MARKS

1. PARTS OF CELL
2. TYPES OF JOINTS
3. CONDUCTION SYSTEM OF HEART
4. MUSCLES OF RESPIRATION
5. NORMAL HR, BP, TEMP
6. TYPES OF BLOOD VESSELS
7. HORMONES PRODUCED BY PITUITARY GLANDS
8. PARTS OF NERVOUS SYSTEM (BOTH CNS AND PNS)
9. TYPES OF BLOOD CELLS
10. WHAT IS CSF? WHAT IS LUMBAR PUNCTURE?
11. PULSE POINTS
12. PARTS OF DIGESTIVE SYSTEM IN ORDER
13. WHAT IS COPD?
14. LIST SPECIAL SENSES
15. TYPES OF MUSCLE TISSUES
16. FUNCTIONS OF URINARY SYSTEM
17. MECHANISM OF VENTILATION
18. PARTS OF BRAIN
19. DIFFERENCE BETWEEN TENDONS AND LIGAMENTS

20. FUNCTIONS OF THE INTEGUMENTARY SYSTEM
21. SYSTEMIC AND PULMONARY CIRCULATION
22. WHAT IS BILE?
23. TYPES OF TISSUES
24. LIST SPINAL AND CRANIAL NERVES
25. DEFINE THE FOLLOWING
    A) HEMOLYSIS
    B) APNEA
    C) HYPOXIA
    D) HOMEOSTASIS

BASICS TO ANESTHETICS – 25 MARKS

1. WHAT IS ANESTHESIA?
2. WHAT IS CRITICAL FLOW?
3. PARTS OF ANESTHESIA MACHINE
4. BOYLE'S LAW
5. WHAT ARE MEDICAL GASES?
6. LIST EQUIPMENTS USED FOR GENERAL ANESTHESIA
7. PHASES OF ANESTHESIA CARE
8. STATES OF MATTER
9. INDICATIONS FOR OXYGEN THERAPY
10. WHAT IS HANGER YOLK ASSEMBLY?
11. PHASES OF GENERAL ANESTHESIA
12. TYPES OF HYPOXIA
13. ASA CLASSIFICATION
14. HAZARDS OF OXYGEN THERAPY
15. PIN INDEX SYSTEM
16. DIFFERENCE BETWEEN SPINAL AND EPIDURAL ANESTHESIA
17. NAME SOME GENERAL ANESTHETIC AGENTS
18. CONSIDERATIONS FOR CHOOSING A SPECIFIC TYPE OF ANESTHESIA
19. STANDARDS FOR BASIC ANESTHESIA MONITORING
20. STORAGE OF MEDICAL GASES
21. OXYGEN DELIVERY METHODS
22. FLUID FLOW TYPES
23. HYPOXEMIA
24. PREOPERATIVE EVALUATION OF ANESTHESIA
25. PREREQUISITES FOR ANESTHESIA

MICROBIOLOGY – 10 MARKS

1. WHAT IS MICROBIOLOGY?
2. WHAT IS IMMUNITY?
3. MICROORGANISMS ARE CLASSIFIED INTO HOW MANY TYPES?
4. TYPES OF IMMUNITY
5. NATURAL AND PASSIVE IMMUNITY
6. DIFFERENT TYPES OF BACTERIAL INFECTIONS
7. WHAT ARE VIRAL DISORDERS?
8. CELLS OF IMMUNE SYSTEM
9. DEFENSE MECHANISM OF NATURAL IMMUNITY
10. DIFFERENTIATE THE BACTERIA BASED ON THEIR CELLMEMBRANE

PATHOLOGY – 15 MARKS

1. WHAT IS PATHOLOGY?
2. WHAT IS PATHOGEN?
3. NEOPLASTIC DISORDERS
4. CARDIOVASCULAR DISORDERS
5. CYSTIC DISORDERS
6. SYMPTOMS OF CYSTIC DISORDERS
7. CAUSES OF MUSCULOSKELETAL DISORDERS
8. ATHEROSCLEROSIS
9. INFLAMMATORY HEART DISEASE
10. OSTEOPOROSIS
11. URINARY TRACT INFECTIONS
12. WHAT ARE SEXUALLY TRANSMITTED DISEASES?
13. VASCULAR DISORDERS
14. SYMPTOMS OF NERVOUS DISORDERS
15. CAUSES OF NEOPLASTIC DISORDERS

SOFT SKILLS – 05 MARKS

1. WHAT ARE INTERPERSONAL SKILLS?
2. SMILING FACE
3. WHAT ARE COMMUNICATION SKILLS?
4. HOW TO MANAGE TIME?
5. RESPONSIBILITY AS A HEALTHCARE PROFESSIONAL

Gram Tarang Employability Training Services (p) ltd.

Clearance Certificate

Employee Name	CHOPPAVARAPU DORCAS	Date of Join	11/05/2018
Reporting To	V. RAVI KUMAR	Date of Leaving	30/11/2018
Designation	ASSOCIATE TRAINER, SOFT SKILLS	Work Location	VIJAYAWADA.

Sl No	Department	Description	Comments	Signature
01	Department Head	Current Pending task	NONE	
		Documents	–	
		Files (Soft & Hard)	–	
02	Accounts & Finance	Company Loan	NA	
		Advance	NA	
		Financial liabilities	NA	
		Bill liabilities	NA	
03	HR	Stationary	–	
		Files	–	
		Company Assets	–	
		Salary	November Pending	
		Login ID	choppavarapu.anand@gramtarang.orgin	
		Laptop & Accessories	–	
04	Store/IT	–	–	

I hereby declared that I have handed over all my charge and company property to all respective departments.

C. Dorcas Anand	29-11-2018	
Employee Signature	**Date**	**Director/HR**

GRAM TARANG EMPLOYABILITY TRAINING SERVICES (GTETS) DATE 29/11 GTETS

WORKSHOP

SENSOR GUIDED ROBOT

DATE: 18-09- 2018 to 19-09-2018

No. of Participants : 47

Instructor: Mr. Tej Kumar Saka,

Engineer at Roboversity, Skyfi Labs

DEPARTMENT OF PHYSICS & ELECTRONICS

SRR & CVR GOVERNMENT DEGREE COLLEGE(A)

MACHAVARAM, VIJAYAWADA

Sensor Guided Robot

Robotics workshop is conducted to Physics & electronics students on 18th & 19th of September month 2018 with the collaboration of Sky- fi labs Bangalore run by IIT Kanpur, alumni affiliated to Angel's Organisation, Bangalore.. They had trained students to impart the skills of mechanics, electronics involved to make robots.

Workshop Outline: Students are trained to develop following projects using Ardunio programming with sensors.

Installation of Arduino Software in laptop & Programming to the Arduino UNO.

Explanation of that how the Programming language is stored in microcontroller as binary instructions.

Electrical Circuit Design for

Line Follower Robot
Obstacle Follower Robot
Obstacle Avoider Robot

Arduino based line follower robot, used IR Transmitters and IR receivers. They are used for sending and receiving light. IR transmits infrared lights. When infrared rays fall on the white surface, it's reflected back and caught by IR sensor which generate some voltage changes.

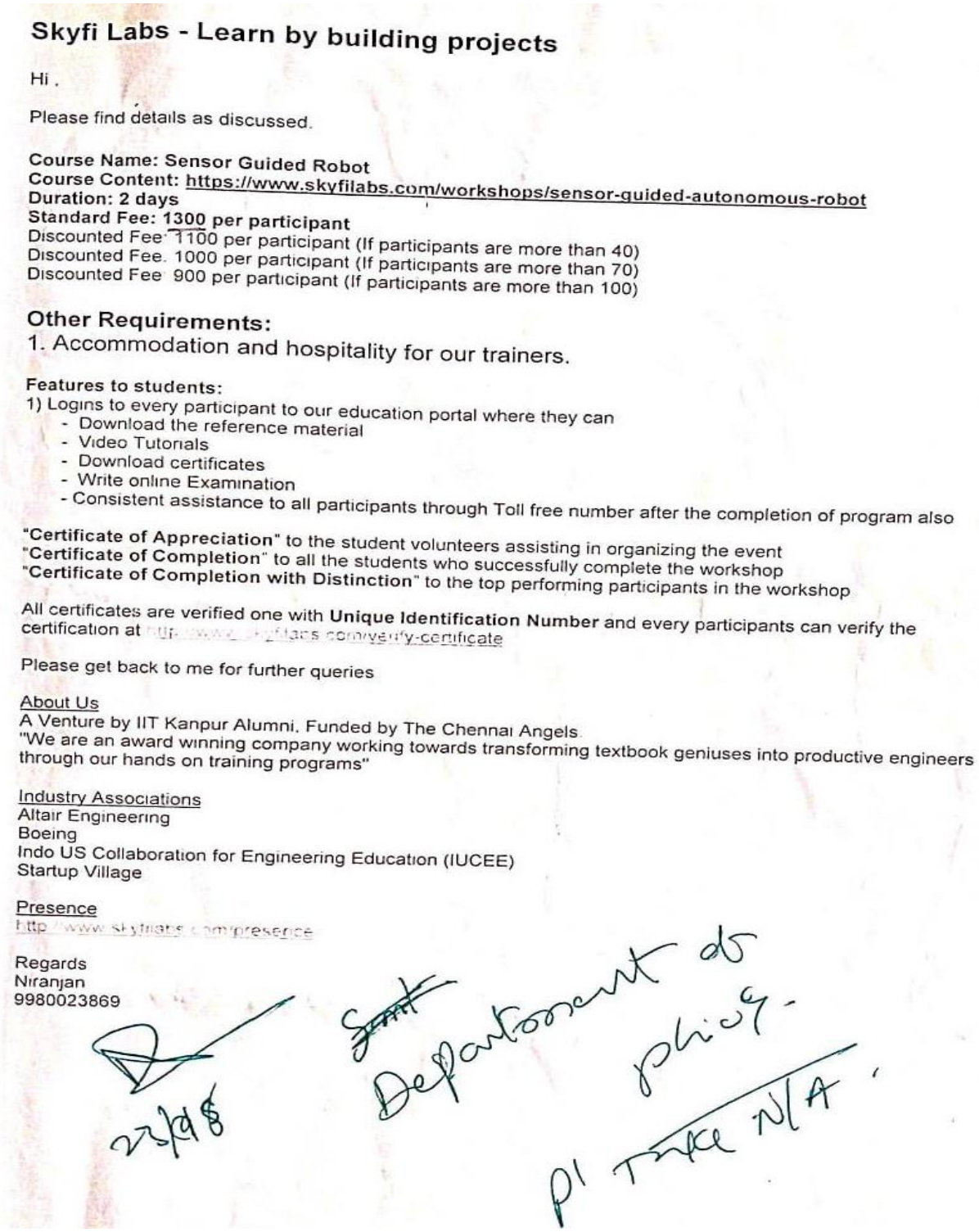
By using ultrasonic sensor, the line follower can detect an obstacle and can stop till the obstacle is removed. These robots can be used as automated equipment carriers in industries replacing traditional conveyer belts.
An obstacle avoiding robot is a fully autonomous robot which can be able to avoid any obstacle which it face when it move.

Action Plan

Department in charge Smt. P. Sailaja & Senior faculty Dr. R. Kameswari actively organised this training by proper Planning. They coordinated this training program with the Skifi Lab team, Bangalore through telephonic conservation & emails.

Skifi Lab accepted to train students & the fee amount to offer the course is sent through email.

Principal Dr. Velaga Joshi readily accepted the proposal forwarded by Department of Physics & Electronics Department & provided financial support required to conduct the Robotics Training. Principal Sir, also provided Computer systems in computer Lab to program the Ardunio.



Skyfi Labs - Learn by building projects

Hi ,

Please find details as discussed.

Course Name: Sensor Guided Robot
Course Content: https://www.skyfilabs.com/workshops/sensor-guided-autonomous-robot
Duration: 2 days
Standard Fee: 1300 per participant
Discounted Fee 1100 per participant (If participants are more than 40)
Discounted Fee. 1000 per participant (If participants are more than 70)
Discounted Fee 900 per participant (If participants are more than 100)

Other Requirements:
1. Accommodation and hospitality for our trainers.

Features to students:
1) Logins to every participant to our education portal where they can
- Download the reference material
- Video Tutorials
- Download certificates
- Write online Examination
- Consistent assistance to all participants through Toll free number after the completion of program also

"**Certificate of Appreciation**" to the student volunteers assisting in organizing the event
"**Certificate of Completion**" to all the students who successfully complete the workshop
"**Certificate of Completion with Distinction**" to the top performing participants in the workshop

All certificates are verified one with **Unique Identification Number** and every participants can verify the certification at http://www.skyfilabs.com/verify-certificate

Please get back to me for further queries

About Us
A Venture by IIT Kanpur Alumni, Funded by The Chennai Angels
"We are an award winning company working towards transforming textbook geniuses into productive engineers through our hands on training programs"

Industry Associations
Altair Engineering
Boeing
Indo US Collaboration for Engineering Education (IUCEE)
Startup Village

Presence
http://www.skyfilabs.com/presence

Regards
Niranjan
9980023869

23/9/8

Department of physics.
PI Trace N/A.

To

Dr.Velaga Joshi

Chairman of the Finance Committee (Autonomous)

SRR & CVR Government Degree College

Vijayawada.

Respected Sir,

Sub: Allotment and issue of check from UGC (Autonomous funds) to conduct Workshop on Robotics on 18th and 19th September 2018 and Projects with PCB formations in December 2018

With reference to the subject, we department of Physics and Electronics have taken permission to conduct workshop on Robotics on 18th & 19th September 2018 in our College with UGC Autonomous funds. We are collaborating with SkyfiLabs, Bangalore. A Venture by IIT Kanpur Alumni. Funded by The Chennai Angels.

Course Name: Sensor Guided Robot
Course Content: https://www.skyfilabs.com/workshops/sensor-guided-autonomous-robot
Duration: 2 days

Certificate of Appreciation" to the student volunteers assisting in organizing the event
"**Certificate of Completion**" to all the students who successfully complete the workshop
"**Certificate of Completion with Distinction**" to the top performing participants in the workshop

All certificates are verified one with **Unique Identification Number** and every participants can verify the certification at http://www.skyfilabs.com/verify_certificate

The Communications from Skyfi Labs, Objective and outcome of the project are attached to this letter. The workshop enables our student to create robotic projects and PCB based projects which carry marks in their internal assessments. Main aim of the Workshop is to give " Hands on " experience to our students in creation of Projects and lead them to skill based learning.

We need an amount of Rs.50,000/- to conduct the two workshops. So kindly direct the UGC Finance committee to arrange the check to conduct the workshop success fully.

Thanking You Sir,

Yours faithfully,

Smt.P.Sailaja
Lecturer in-charge
Dept. of Physics & Electronics
SRR & CVR Govt. Degree College
VIJAYAWADA-4

(In-charge of the Dept)

केनरा बैंक Canara Bank
VIJAYAWADA GOVERNORPET Branch
VIJAYAWADA ANDHRA PRADESH 520002
IFSC : CNRB0000680
2018/PSSKW
MULTI-CITY SB
Valid for three months only from the date of instrument
1 9 0 9 2 0 1 8
D D M M Y Y Y Y

Pay Skyfi Education labs pvt ltd
या धारक को Or Bearer
Rupees रुपये Fourty nine thousand five hundred Rupees only.
अदा करें ₹ 49,500/-
A/c. No. 0680101022174
Payable at par at all our branches in India
PRINCIPAL
SRR& CVR GOVT. DEGREE COLLEGE
(Autonomus)
Machavaram, VIJAYAWADA - 520 004.

Financial Support:

The financial support from UGC autonomous funds made this dream true. A cheque of Rs. 49,500 is paid to the Skyfi Education Labs, Pvt. Ltd by the SRR & CVR Government Degree college to get train Students in advanced Robotics.

To

Dr.Velaga Joshi

PRINCIPAL

SRR & CVR Government Degree College

Vijayawada.

Respected Sir,

Sub: Permission for Computer systems along with Computer Lab to conduct Workshop on Robotics on 18th and 19th September 2018

With reference to the subject, we department of Physics and Electronics have taken permission to conduct workshop on Robotics on 18th & 19th September 2018 in our College with UGC Autonomous funds. We are collaborating with SkyfiLabs, Banglore. They requested for 10 Computer Systems with High speed internet in Computer Lab itself, Smart board. So kindly direct the Computer Science Department to make the necessary arrangements to give the systems with internet and Windows 7.

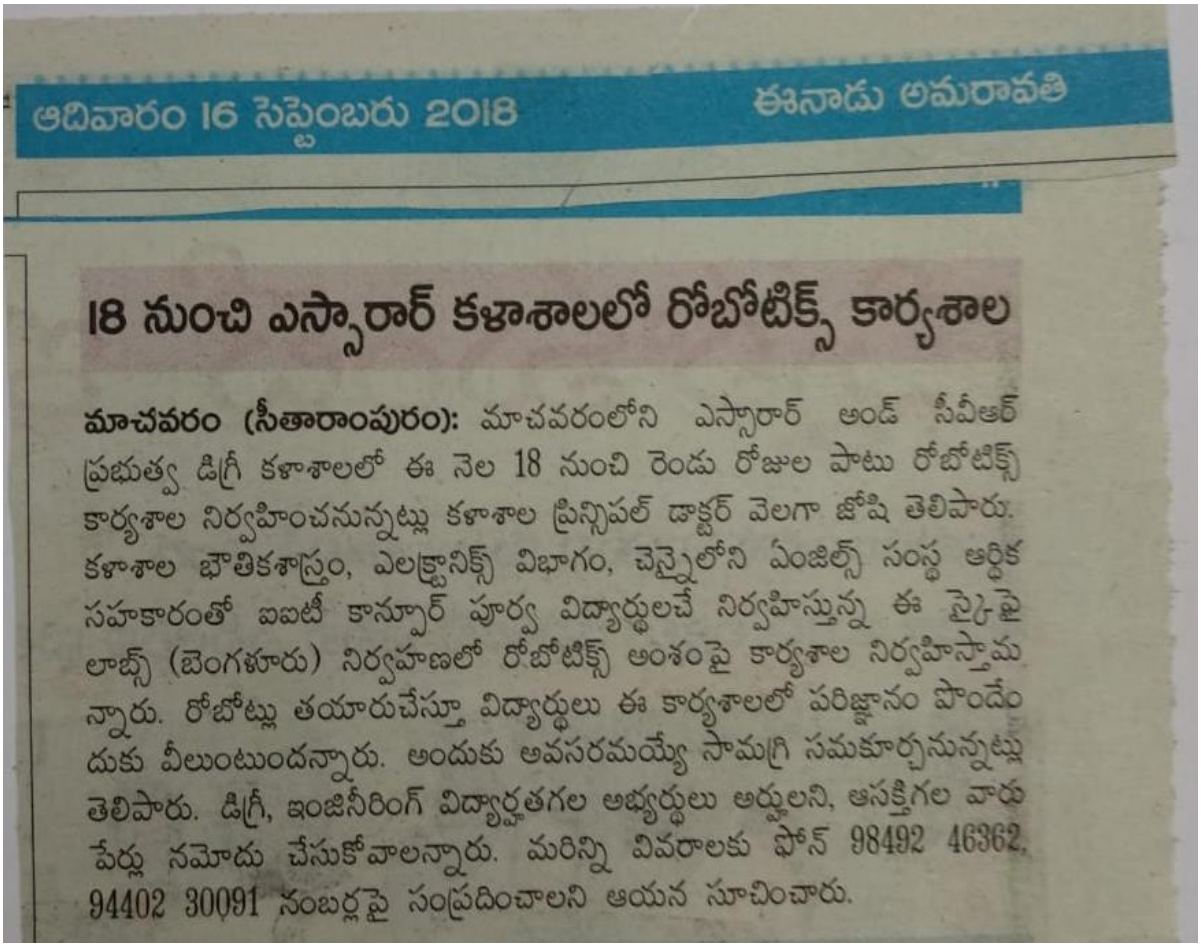
Thanking You Sir,

Yours faithfully,

P. Sailaja

(Smt.P.Sailaja)

(Incharge of the Dept)



ఆదివారం 16 సెప్టెంబరు 2018 ఈనాడు అమరావతి

18 నుంచి ఎస్సారార్ కళాశాలలో రోబోటిక్స్ కార్యశాల

మాచవరం (సీతారాంపురం): మాచవరంలోని ఎస్సారార్ అండ్ సీవీఆర్ ప్రభుత్వ డిగ్రీ కళాశాలలో ఈ నెల 18 నుంచి రెండు రోజుల పాటు రోబోటిక్స్ కార్యశాల నిర్వహించనున్నట్లు కళాశాల ప్రిన్సిపల్ డాక్టర్ వెలగా జోషి తెలిపారు. కళాశాల భౌతికశాస్త్రం, ఎలక్ట్రానిక్స్ విభాగం, చెన్నైలోని ఏంజిల్స్ సంస్థ ఆర్థిక సహకారంతో ఐఐటీ కాన్పూర్ పూర్వ విద్యార్థులచే నిర్వహిస్తున్న ఈ స్కైఫై లాబ్స్ (బెంగళూరు) నిర్వహణలో రోబోటిక్స్ అంశంపై కార్యశాల నిర్వహిస్తామన్నారు. రోబోట్లు తయారుచేస్తూ విద్యార్థులు ఈ కార్యశాలలో పరిజ్ఞానం పొందేందుకు వీలుంటుందన్నారు. అందుకు అవసరమయ్యే సామగ్రి సమకూర్చనున్నట్లు తెలిపారు. డిగ్రీ, ఇంజినీరింగ్ విద్యార్హతగల అభ్యర్థులు అర్హులని, ఆసక్తిగల వారు పేర్లు నమోదు చేసుకోవాలన్నారు. మరిన్ని వివరాలకు ఫోన్ 98492 46362, 94402 30091 నంబర్లపై సంప్రదించాలని ఆయన సూచించారు.

Learning Process

Interested students were selected from Science streams.30 students from III B.Sc , II B.Sc (MPCs, MECs) had enthusiastically participated in this workshop.

SRR & CVR GOVERNMENT DEGREE COLLEGE (A)

MACHAVARAM, VIJAYAWADA

TWO DAYS WORKSHOP ON ROBOTICS

September 18th & 19th

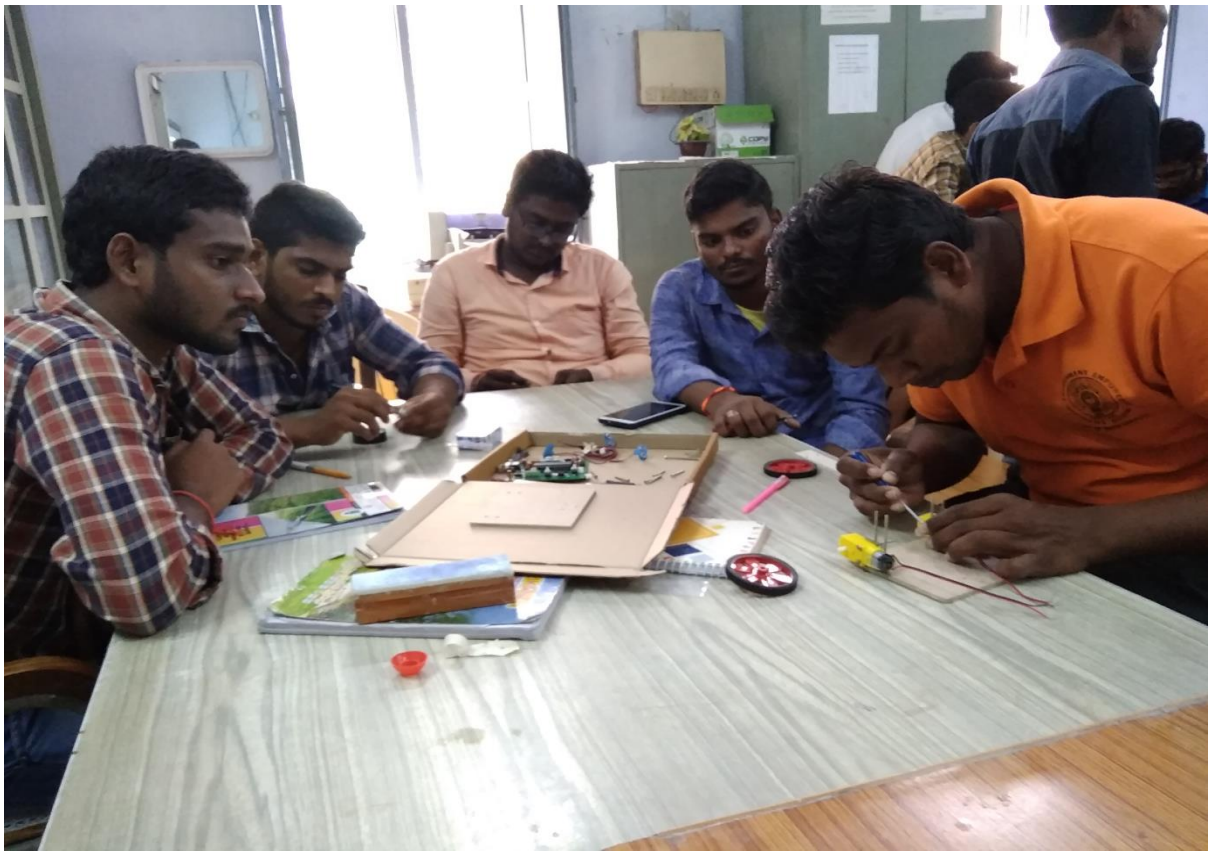
S.NO	NAME OF THE STUDENT	CLASS	COLLEGE NAME	E MAIL ID	MOBILE NO
1	E VENKATESWARA RAO	III BSC	SRR &CVR DEGREE COLLEGE	vaenkyerlla@gmail.com	7989583634
2	M SANDHYA	III BSC	SRR &CVR DEGREE COLLEGE	Sandhyasandi0001@gmail.com	7286813133
3	CH VASU	III BSC	SRR &CVR DEGREE COLLEGE	Vasuvicky00@gmail.com	7893343876
4	G S PRITHVI SAI	III BSC	SRR &CVR DEGREE COLLEGE	Prithvisai24@gmail.com	9493871663
5	D LOK MANOHAR	III BSC	SRR &CVR DEGREE COLLEGE	Lokmanoharkumar20@gmail.com	9701366639
6	R DURGA PRASAD	III BSC	SRR &CVR DEGREE COLLEGE	r.durgaprasad@gmail.com dsp903220	8885910208
7	V KALYAN KUMAR	III BSC	SRR &CVR DEGREE COLLEGE	kalyankumarkick@gmail.com	7794085191
8	T VENKATA SAI	III BSC	SRR &CVR DEGREE COLLEGE	Saivenkat71303@gmail.com	8886089120
9	M VIKRAM	III BSC	SRR &CVR DEGREE COLLEGE	Medavikram2017@gmail.com	9963192656
10	S SAI BHARGAV	III BSC	SRR &CVR DEGREE COLLEGE	Sbhargav626@gmail.com	8919991553
11	K NANDHINI	III BSC	SRR &CVR DEGREE COLLEGE	Nandhunandini79@gmail.com	9948809396

S.NO	NAME OF THE STUDENT	CLASS	COLLEGE NAME	E MAIL ID	MOBILE NO
12	J NIKHILA	III BSC	SRR &CVR DEGREE COLLEGE	Jonnalanikhila98@gmail.com	8328586453
13	CH VENKATA GANESH	III BSC	SRR &CVR DEGREE COLLEGE	Chintalaboinaganesh386@gmail.com	8464878023
14	D HANUMAN SANKAR	III BSC	SRR &CVR DEGREE COLLEGE	Hanumansankar57@gmail.com	9553404259
15	J BHASKAR	III BSC	SRR &CVR DEGREE COLLEGE	jonnakuribala@gmail.com	7285930718
16	P HARI KRISHNA	III BSC	SRR &CVR DEGREE COLLEGE	Haripydi5678@gmail.com	8179231510
17	K SUDHA RANI	III BSC	SRR &CVR DEGREE COLLEGE	Sudha99rani@gmail.com	9581508120
18	P JYOTSNA DEVI	III BSC	SRR &CVR DEGREE COLLEGE	Jyothsnadevi987@gmail.com	9154224493
19	G SUSMITHA	III BSC	SRR &CVR DEGREE COLLEGE	Susmithag95@gmail.com	9963996140
20	G HEMA LATHA	III BSC	SRR &CVR DEGREE COLLEGE	Hemadolly98@gmail.com	7901249772
21	D NEERAJA	III BSC	SRR &CVR DEGREE COLLEGE	Neerajadhanekula777@gmail.com	7095741676
22	M AKSHAY PAUL	I BSC	SRR &CVR DEGREE COLLEGE	bunnyakshaypaul@gmail.com	7036124373
23	S DINESH VENKATA SRI	III BSC	SRR &CVR DEGREE COLLEGE	Dineshnaidu48@gmail.com	7382325466
24	B SURYA PRAKASH	III BSC	SRR &CVR DEGREE COLLEGE	busasuryaprakash@gmail.com	9912824361
25	G SRI LAKSHMI	II BSC	SRR &CVR DEGREE COLLEGE	Srilakshmigaddam1234@gmail.com	8142491435
26	G DURGA BHAVANI	II BSC	SRR &CVR DEGREE COLLEGE	Durga9102000@gmail.com	9052882942
27	CH YASASVI	II BSC	SRR &CVR DEGREE COLLEGE	chintayasasvi@gmail.com	7288927266
28	S SATISH	III BSC	SRR &CVR DEGREE COLLEGE	Satish7prince@gmail.com	7032326993
29	K RAJESH	III BSC	SRR &CVR DEGREE COLLEGE	Katikalarajesh927@gmail.com	9553266856
30	R SITA MAHA LAKSHMI	II BSC	SRR &CVR DEGREE COLLEGE	Rseetha9k@gmail.com	8106175336

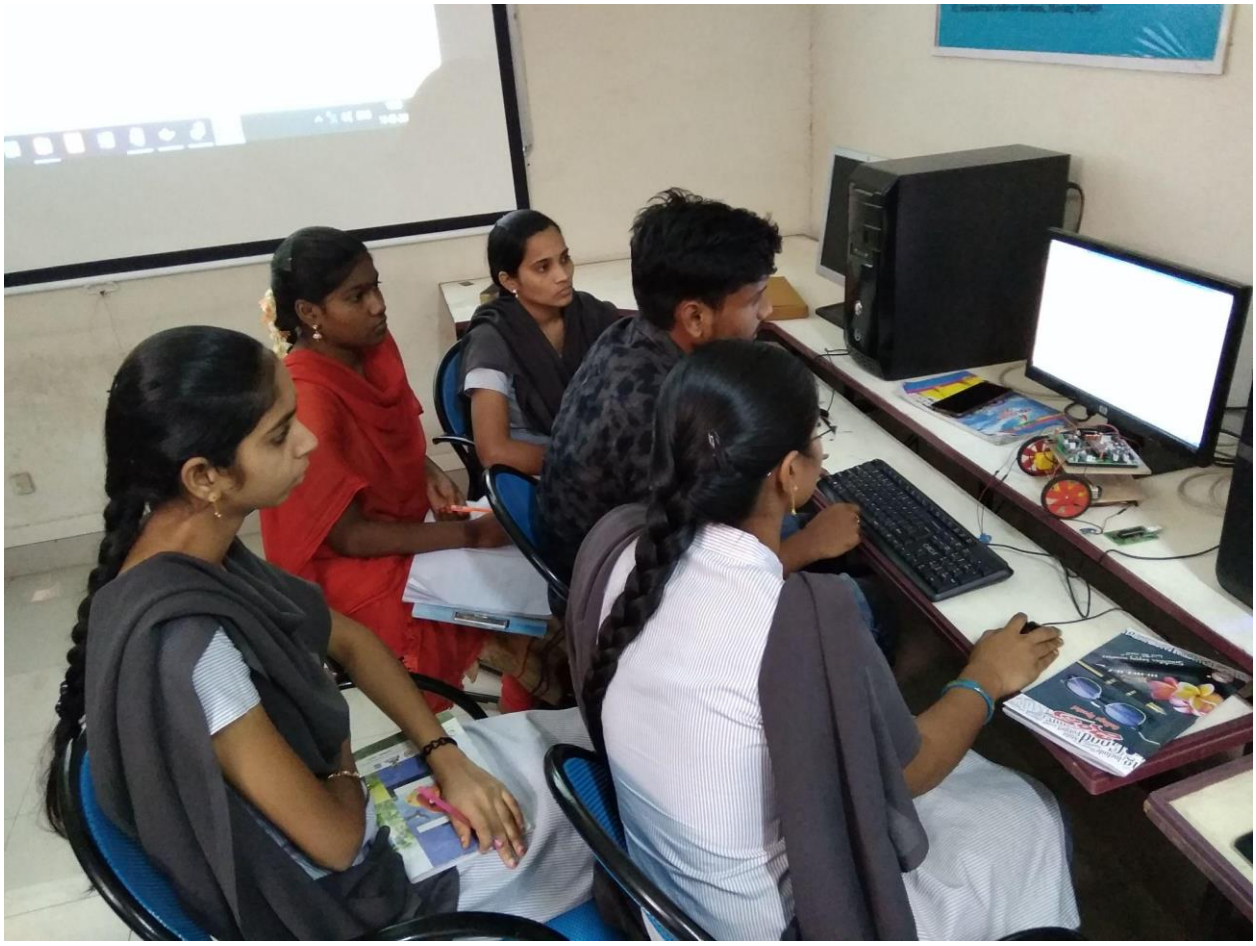
S.NO	NAME OF THE STUDENT	CLASS	COLLEGE NAME	E MAIL ID	MOBILE NO
31	S PUJITHA	II BSC	SRR &CVR DEGREE COLLEGE	poojithasanagasetti@gmail.com	9110562198
32	P SUREKHA	III BSC	SRR &CVR DEGREE COLLEGE	Pallipamusurekha98@gmail.com	9133436624
33	V RAJA NAIK	III BSC	SRR &CVR DEGREE COLLEGE	Naik3550@gmail.com	9133029432
34	L SAI KUMAR	III BSC	SRR &CVR DEGREE COLLEGE	Landasaikumar09@gmail.com	8639638588
35	MD MEHRAJUNNISA	III BSC	SRR &CVR DEGREE COLLEGE	Meharmehraj18@gmail.com	9550401265
36	CH PRATYUSHA	II BSC	SRR &CVR DEGREE COLLEGE	Chekurthipati1998@gmail.com	9505946291
37	K GOPI RAJU	III BSC	SRR &CVR DEGREE COLLEGE	Gopirajukorra0007@gmail.com	8688457573
38	N SAI MANEESA	III BSC	SRR &CVR DEGREE COLLEGE	Nsaimaneesha1988@gmail.com	7095165464
39	R MANI KANTA	III BSC	SRR &CVR DEGREE COLLEGE	Manikanta95r@gmail.com	7095387917
40	B V VARDHAN NAIDU	B TECH	SIR C R REDDY ENGG COLLEGE	Vivekavardhan12091999@gmail.com	9440231192
41	J DURGA PRASANTH	B TECH	SIR C R REDDY ENGG COLLEGE	jakkudurgaprasanth@gmail.com	6303838415
42	N MOHAN KUMAR	III BSC	SRR &CVR DEGREE COLLEGE	Nikkam.mohann599@gmail.com	8885833602
43	P SAMPATH	B TECH	NRI ENGG COLLEGE	Sampathprattipati5@gmail.com	9133219714
44	P A P BAGAT CHOWDARY	B TECH	NRI ENGG COLLEGE	Abishekchowdary434@gmail.com	9573662404
45	M GOPAL LAKSHMAN	B TECH	NRI ENGG COLLEGE	Lakshmanmarupilla1503@gmail.com	9491347438
46	U SURESH	III BSC	SRR &CVR DEGREE COLLEGE	Sureshu589@gmail.com	8522827882
47	J HEMANTH	III BSC	SRR &CVR DEGREE COLLEGE	Hemanthj7595@gmail.com	9700680652

5 Students from NRI, Sir CR Reddy Engineering Colleges also joined in this workshop.

Students were trained theoretically about the programming concepts & had hands on experience to connect electrical circuit to Arduino pins.




S.R.R. & C.V.R. Government Degree College
UGC SPONSORED (AUTONOMOUS)
WORKSHOP ON ROBOTICS
"LEARN BY BUILDING PROJECTS"
Dr. VELAGA JOSHI



SRR & CVR GOVERNMENT DEGREE COLLEGE (A)

MACHAVARAM, VIJAYAWADA

TWO DAYS WORKSHOP ON ROBOTICS

September 18th & 19th

S NO	NAME OF THE STUDENT	18-SEPTEMBER		19 SEPTEMBER	
		FN	AN	FN	AN
1	E VENKATESWARA RAO				
2	M SANDHYA				
3	CH VASU				
4	G S PRITHVI SAI				
5	D LOK MANOHAR				
6	R DURGA PRASAD				
7	V KALYAN KUMAR				
8	T VENKATA SAI				
9	M VIKRAM				
10	S SAI BHARGAV				
11	K NANDHINI				
12	J NIKHILA				
13	CH VENKATA GANESH				
14	D HANUMAN SANKAR				
15	J BHASKAR				
16	P HARI KRISHNA				
17	K SUDHA RANI				
18	P JYOTSNA DEVI				
19	G SUSMITHA				
20	G HEMA LATHA				
21	D NEERAJA				
22	M AKSHAY PAUL				

23	S DINESH VENKATA SRI				
24	B SURYA PRAKASH				
25	G SRI LAKSHMI				
26	G DURGA BHAVANI				
27	CH YASASVI				
28	S SATISH				
29	K RAJESH				
30	R SITA MAHA LAKSHMI				
31	S PUJITHA				
32	P SUREKHA				
33	V RAJA NAIK				
34	L SAI KUMAR				
35	MD MEHRAJUNNISA				
36	CH PRATYUSHA				
37	K GOPI RAJU				
38	N SAI MANEESA				
39	R MANI KANTA				
40	B V VARDHAN NAIDU				
41	J DURGA PRASANTH				
42	N MOHAN KUMAR				
43	P SAMPATH				
44	P A P BAGAT CHOWDARY				
45	M GOPAL LAKSHMAN				
46	U SURESH				
47	J HEMANTH				

S.R.R & C.V.R Govt. Degree College (Autonomous)

Machavaram, Vijayawada-520 004, Krishna Dt. A.P

TWO DAYS WORKSHOP ON " ROBOTICS"

18-09-2018 & 19-09-2018, From 9.30 am to 5.00 pm

Conducted By

DEPARTMENT OF PHYSICS & ELECTRONICS

***in collaboration with* SKYFI LABS, Bangalore, A venture by IIT Kanpur Alumni,**

Funded by the Chennai Angels

REGISTRATION FORM

1. Name of the Applicant : J. Nikhila
2. Father's Name : J. Suresh
3. Date of Birth & Age : 27-09-1998
4. Address for Communication : DR.No. 32-26-62
   Sunkanavari street
   Machavaram. vijayawada
   Mobile No: 8328586453
   E-Mail ID: Jannalanikhila98@gmail.com
5. Educational Qualifications : Degree final year (pursuing)
6. Aadhar No. : 971040934245

7. Declaration: I hereby declare that the Information furnished above is ture to the the best of my knoledge. I will follow the rules and regulations of the College.

Date: - - 2018.

J. Nikhila

Signature of the Candidate

For Office Use Only

Fee paid Rs.....(Rupees.....only)

Vide Receipt No. : ....., Dt. - - 2018.

Office Staff **Incharge of Dept.** **Principal**

S.R.R & C.V.R Govt. Degree College (Autonomous)

Machavaram, Vijayawada-520 004, Krishna Dt. A.P

TWO DAYS WORKSHOP ON " ROBOTICS"

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DEPARTMENT OF PHYSICS & ELECTRONICS

***in collaboration with* SKYFI LABS, Bangalore, A venture by IIT Kanpur Alumni,**

Funded by the Chennai Angels

REGISTRATION FORM

1. Name of the Applicant : G. Hemalatha
2. Father's Name : G. Srinadh
3. Date of Birth & Age : 23-7-1998, Age :-20y
4. Address for Communication : Sing nagar D.No:-77-137-1
   Sundharaiah nagar 4th Line
   Payakapuram, Vijayawada
   Mobile No: 7901204772
   E-Mail ID: Hemadolly98@gmail.com
5. Educational Qualifications : Degree 3rd year B.Sc.
6. Aadhar No. : 996887670984

7. Declaration: I hereby declare that the Information furnished above is ture to the the best of my knoledge. I will follow the rules and regulations of the College.

Date: 18-9-2018.

G. Hemalatha
Signature of the Candidate

For Office Use Only

Fee paid Rs.....(Rupees.....only)

Vide Receipt No. : ....., Dt. - - 2018.

Office Staff **Incharge of Dept.** **Principal**

Learning outcome:

Hands-on experience to students in creation of line follower Robot, obstacle avoiding Robot, obstacle follower robot using Ardunio coding. This leads them to skill based learning.

Students understand the structure and programming of microcontrollers and the techniques.

By using the same sensors and Learners enjoyed changing the programme login using the same sensor and how the function of Robot be modified .

Student participants come up with new ideas for further improvement of robots.

Impact:

The programme gave clear practical experience to students to understand the sensors and programming them with their own logic .

Students are able to develop the sensor Robots.

They got a way to opt Robotics as one of their career opportunities.

Students felt this course was fun with Knowledge.

Conclusion

Learners gave very positive feedback on this programme.

They got great satisfaction from designing their own robot with programming.

Students are issued digital verified e- certificates to their emails from Skifi labs, Bangalore.


VERIFIED CERTIFICATE

Skyfi labs
LEARN ENGINEERING. PRACTICALLY!

Roboversity

www.roboversity.com
www.skyfilabs.com

19 September 2018

SRR & CVR Govt. Degree College, Vijayawada

This is to certify that

SAILAJA PONNAM

has completed a 2-day project-based training program on

Sensor Guided Robotics

Unique Certificate Code: **48430044**

Verify at www.skyfilabs.com/verify-certificate/48430044

Pavan Kumar P
CEO, Skyfi Labs

SRR & CVR GOVERNMENT DEGREE COLLEGE (A)

MACHAVARAM, VIJAYAWADA

TWO DAYS WORKSHOP ON ROBOTICS

September 18th & 19th-2018

FEEDBACK FORM

1. **Name of the Trainee:** E. Venkateswara Rao
2. **Name of the College:** SRR & CVR Govt Degree college Vja(A)
3. **email-id:** : vaenkyesrlla@gmail.com
4. **How useful was workshop?**

(a) Beyond expectations (b) Satisfactory (c) Moderate

5. **How would you rate length of sessions?**

(a) Too long (b) Just right (c) Too short

6. **How would you rate this workshop?**

(a)Excellent (b) Very good (c) Good

7. **Trainer's knowledge of the subject**

(a) Excellent (b) Very good (c) Good

8. **Course materials/booklets**

(a) Excellent (b) Very good (c) Good

9. **The facilities**

(a) Excellent (b) Very good (c) Good

10. **Would you recommend this course to your friends?**

(a)Yes (b) No (c) Not sure

SRR & CVR GOVERNMENT DEGREE COLLEGE (A)

MACHAVARAM, VIJAYAWADA

TWO DAYS WORKSHOP ON ROBOTICS

September 18th & 19th-2018

FEEDBACK FORM

1. **Name of the Trainee:** E. Venkateswara Rao
2. **Name of the College:** SRR & CVR Govt Degree college Vja(
3. **email-id:** : Vaenkyeswa@gmail.com
4. **How useful was workshop?**

(a) Beyond expectations (b) Satisfactory (c) Moderate

5. **How would you rate length of sessions?**

(a) Too long (b) Just right (c) Too short

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9. **The facilities**

(a) Excellent (b) Very good (c) Good

10. **Would you recommend this course to your friends?**

(a)Yes (b) No (c) Not sure

ఈనాడు అమరావతి

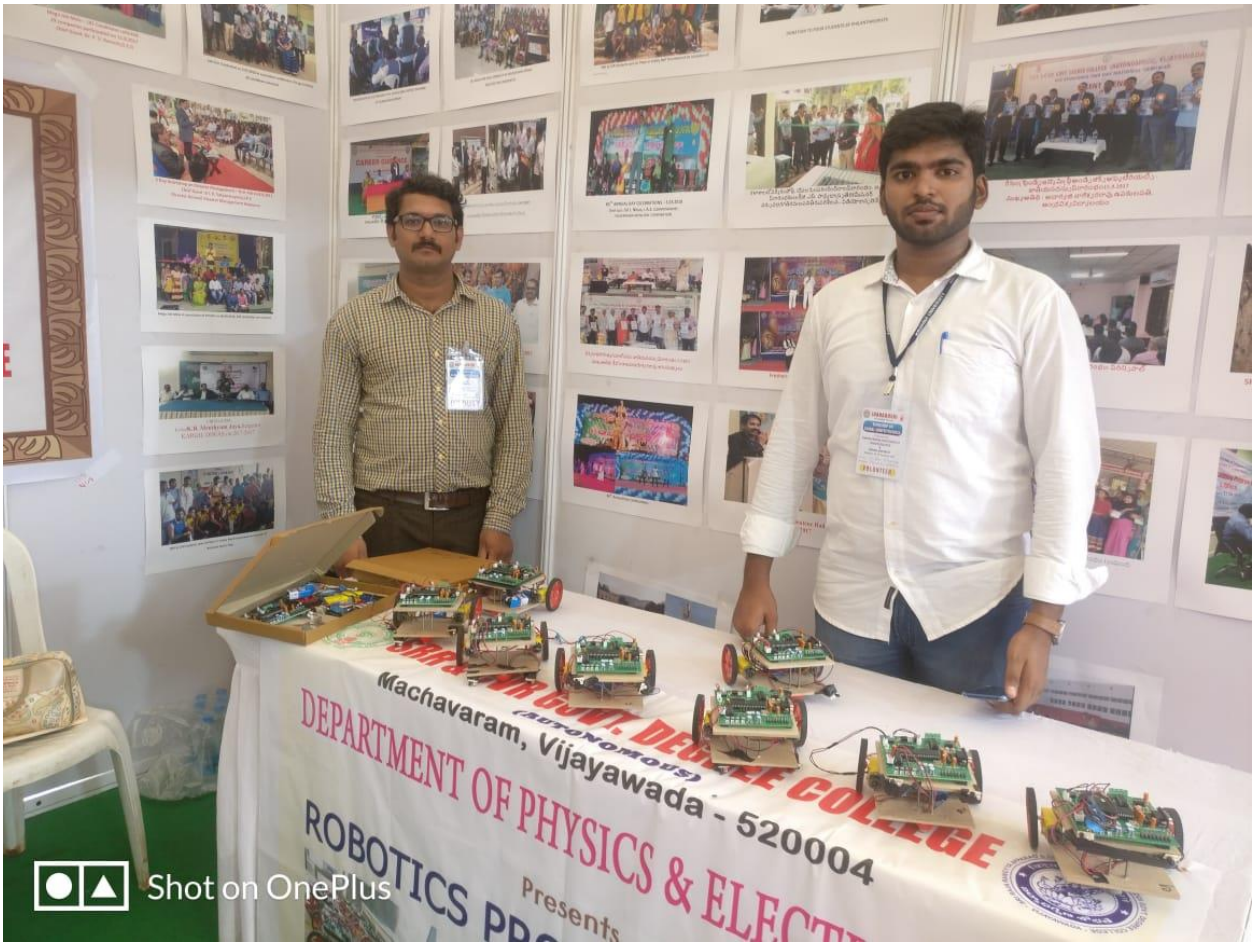
గురువారం 20 సెప్టెంబరు 2018 .. 20 పేజీలు

ముగిసిన రోబోటిక్స్ కార్యశాల

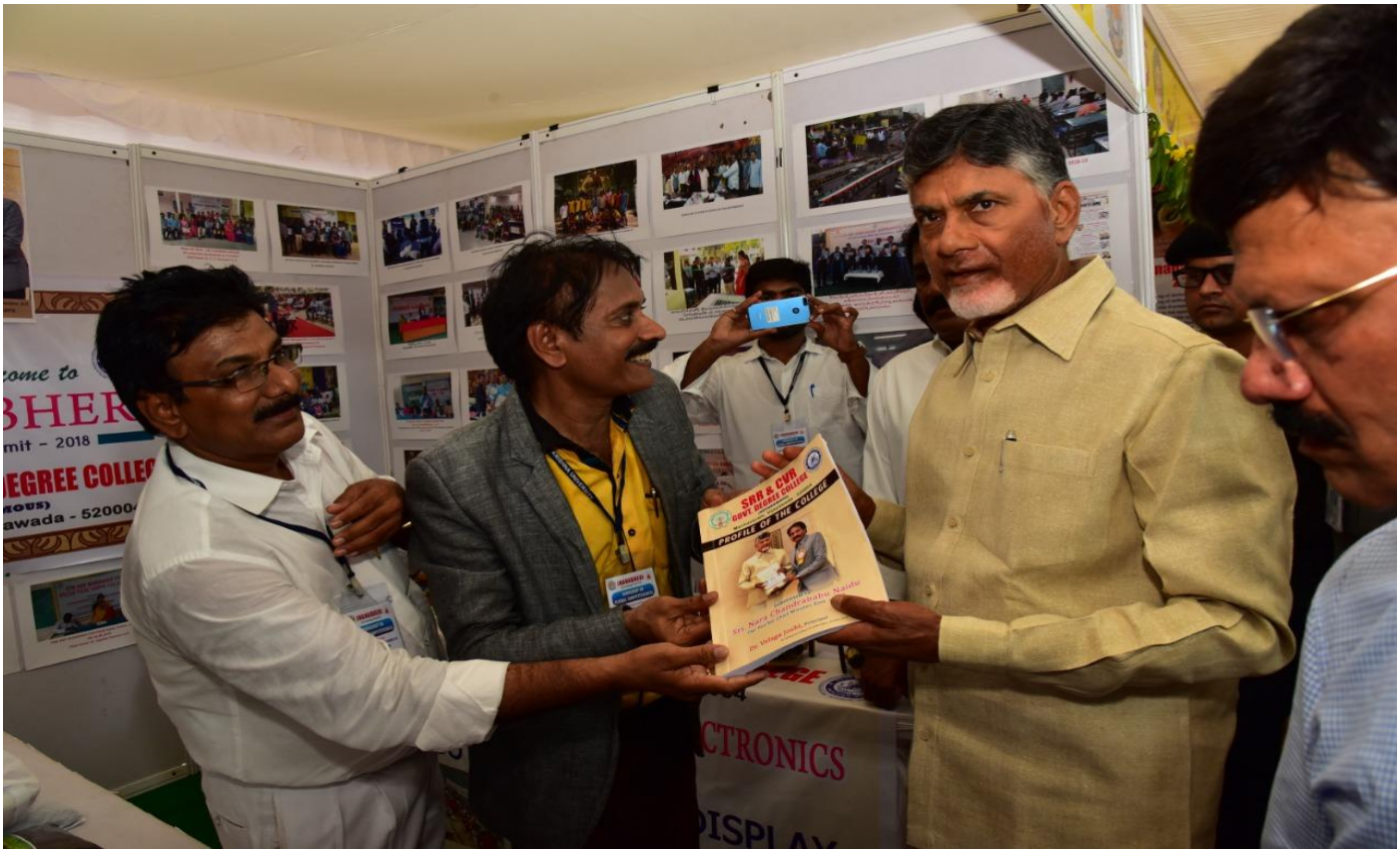
మాచవరం (సీతారాంపురం, న్యూస్‌టుడే): మాచవరంలోని ఎస్సారార్ అండ్ సీవీఆర్ ప్రభుత్వ డిగ్రీ కళాశాల ఫిజిక్స్, ఎలక్ట్రానిక్స్ విభాగం ఆధ్వర్యంలో రెండు రోజుల పాటు కళాశాలలో నిర్వహించిన రోబోటిక్స్ కార్యశాల బుధవారం ముగిసింది. ఎస్సారార్ అండ్ సీవీఆర్ ప్రభుత్వ డిగ్రీ కళాశాల బీఎస్సీ ఫైనల్ ఇయర్ విద్యార్థులతో పాటు ఇతర డిగ్రీ, ఇంజినీరింగ్ కళాశాలల నుంచి వచ్చిన 55 మంది అభ్యర్థులు కార్యశాలలో పాల్గొని ప్రత్యక్షంగా రోబోట్ తయారీ పరిజ్ఞానాన్ని పొందడమే కాకుండా స్వయంగా రోబోట్లను తయారుచేసి ప్రదర్శించారు. ఈ రోబోట్ల తయారీ కార్యశాలకు ఇంజినీరుగా తేజ్‌కుమార్ వ్యవహరించారు. చెన్నై ఏంజెల్స్ సంస్థచే ప్రాయోజితమైన స్కైపై ల్యాబ్స్ తరపున హాజరై రోబోట్ల తయారీ, పని తీరును విద్యార్థులకు నేర్పించారు. ఎస్సారార్ అండ్ సీవీఆర్ ప్రభుత్వ డిగ్రీ కళాశాల ప్రిన్సిపల్ డాక్టర్ వెలగా జోషి, భౌతికశాస్త్ర విభాగాధిపతి పి.శైలజ, సమన్వయకర్త డాక్టర్ ఆర్.కామేశ్వరి, డాక్టర్ సుజాత, బలరామ్, ఎండీ ఇక్బాల్ పాష, నయోమి పాల్గొన్నారు.

రోబోట్లను తయారుచేసిన విద్యార్థులతో అతిథులు

Students exhibited their robots at Gnanaberi program which is held to encourage innovation skills among the students in the state. The Chief Minister Nara Chandra babu Naidu visited Robotics stall, interacted and appreciated the student's creativity. Honourable APCCE Madam Sujatha Sarma also visited the stall & appreciated the efforts of students.


Machavaram, Vijayawada - 520004
DEPARTMENT OF PHYSICS & ELECTR
ROBOTICS
Presents
Shot on OnePlus

https://www.thehansindia.com/posts/index/Andhra-Pradesh/2018-08-21/Chandrababu-Naidu-to-take-part-in-Gnana-Bheri-on-August-23/406759



Scanned copy of Robotics Workshop Report