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Evaluation Of The Efficacy Of Sevelamer Hydrochloride And Sevelamer Carbonate Together With Dietary Sources In Hyperphosphatemia Condition.

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ABSTRACT

Sevelamer is a phosphate binder that is given as a medication to treat hyperphosphatemia in patients suffering from chronic kidney disorder. It binds to the dietary proteins and prevents the absorption of the same in the body. It is mainly marketed in two forms- hydrochloride form and carbonate form. In the present investigation, the effect of sevelamer hydrochloride and sevelamer carbonate along with the samples of plant extraction was studied on the levels of phosphate absorption in the intestine by the process of dialysis. The result indicated a better absorption capacity of carbonate form of the drug when compared to the hydrocholoride form of the drug. The difference in the absorption was marginally high. Also, we observed that there was a variation in the absorption capacity of the 2 drug forms in combination with the dietary samples. The result indicated that bitter gourd with carbonate form showed the highest absorption for phosphate.

Keywords: hyperphosphatemia, calcification, chronic kidney disorder, sevelamer.

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INTRODUCTION

Dialysis is a major alternative for patients suffering from kidney disease as it is known to be an efficient method of purification of blood. This process as mentioned usually is conducted thrice in a week and hence there can be accumulation of minerals or other elements. This can affect the patient by causing toxicity. One such common problem in patients suffering from chronic kidney disorder (CKD) is hyperphosphatemia [1].

Overabundance measure of phosphate in the body can bring about imbalance of electrolytes prompting to different complications. This happens because of the failure of kidney to work. Normal phosphate level in a typical human is 0.81mmol/L to 1.45mmol/L. Either diseases cause hyperphosphatemia or irregular metabolism of phosphate causing diseases such and chronic kidney disorder, mineral and bone disorder. Hyperphosphatemia also causes secondary hyperparathyroidism and hypocalcemia [2].

Hypocalcemia is a condition where accessibility of calcium for ingestion decreases. Ordinary serum calcium level changes from 2.1mmol/L to 2.6mmol/L. Less than 2.1 mmol/L is named as hypocalcemic condition [3]. Different reasons like pancreatitis, overdose of calcium blockers, and so forth causes hypocalcemic condition. In circumstances where hyperphosphatemia causes this condition, phosphate binds to calcium and accumulates in the tissue. This causes calcification in the veins. In patients experiencing CKD, along with lack of ability to function, calcification prompts to the putrefaction of the tissue because of deficient blood stream [4].

These conditions make it necessary to decrease the levels of the phosphate in the system for which phosphate binders are used. Sevelamer is a commercially available drug that removes phosphate and is recommended for CKD patients as they are prone to calcification [5]. Calcium based sevelamer is avoided for patients suffering from hypocalcemia, hyperparathyroidism, etc. [6]. Dietary aspect is also necessary to maintain the levels of phosphate and other minerals. Therefore a strict diet has to be maintained.

Yongsheng Yang et al., have reported that there is a variation in the absorption capacity of the drug as they differ in their affinity [7]. Therefore, the present work is an attempt to understand the effect of the drug and its absorption capacity as they differ in their affinity. In the present investigation, we seek to provide scientific proof for home remedy recommendation for patients suffering from CKD.

MATERIALS AND METHODS

Plant Source: Plantain stem (*Musa paradisiaca),* Bitter gourd (*Momordicacarantia)*, Chow-Chow (*Sechiumedule)*, Flax seeds (*Linumusitatissimum)*, Sunflower seeds (*Helianthus annus)*, Water melon seeds (*Citrulluslanatus)* was obtained from Nilgiri's Supermarket, Bengaluru, Karnataka, India.

Drug Source: Sevelamer Hydrochloride and Sevelamer Carbonate was obtained from Balaji Medical store, Bengaluru, Karnataka, India.

Source Of Clinical Samples:

Human Dialysed Sample Source: The dialysed sample was obtained from Bangalore Hospital, Bengaluru. The sample was stored in a container and was used for further analysis.

Human blood serum: Blood sample was collected and was added into a centrifuge tube.

EDTA was added and centrifuged at 2000rpm for 5 mins in order to obtain serum.

Equipment: The equipment used were UV-Vis spectrophotometer (Model no.117), Calorimeter (ELICO Ltd., Hyderabad, India. / Model no.CL-157), Centrifuge (REMI laboratory, Maharashtra, India), Pig's intestine as dialysis membrane (*Sus domesticus'*sintestine), weighing balance (Shimadzu/ Model no. ELB300), hot plate (TARSONS Product Pvt Ltd.), magnetic stirrer (REMI Laboratory, Maharashtra, India) and Microwave.

Dialysis membrane source (*Sus domesticus's* intestine): The intestine was obtained from the slaughter house of Bangalore Ham Shop, Benson Town, Bengaluru. The intestine was washed using milliQ water and was used for further analysis.

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Chemicals:

Acetone, methanol, diethyl ether, hydrochloric acid, di-potassium hydrogen ortho-phosphate, calcium phosphate, glacial acetic acid, ammonium molybdate, sodium meta bisulphate (Fisher Scientific India Pvt Ltd., Mumbai, India.), 4-methylaminophenol sulphate (LobaChemie Pvt Ltd., Mumbai, India).

Preparation of plant extract:

The 6 plant samples that were selected, for each of these samples organic and buffer extractions were prepared, resulting in 12 samples. For the organic extracts acetone, diethyl ether, methanol was used for preparing solutions of different concentrations for plant extracts of *viz*; 5%, 20%, 50%, resulting in 54 organic extractions of various concentrations. The 6 plant origin samples were used to prepare 2 buffer extracts using phosphate buffer and bicarbonate buffer of different concentrations *viz*: 5%, 20%, 50%, resulting in 36 buffer extractions. These extracts were further analysed for bioactive molecules using a UV- Vis spectrophotometer at different wavelengths.

Dialysis :

Portions of the intestine were taken. The one end of each portion was tied using a twine and 10.0ml of different combinations of solutions was poured into the lumen of the intestine through the other end and was tied. This portion of intestine acted as a dialysis membrane. This membrane was placed in a beaker containing 50.0ml MilliQ water; 1.0ml of the same solution was taken into a test tube to estimate the amount of phosphate before dialysis. Then this beaker was placed on a magnetic stirrer for 30 mins. From the eluent obtained after 30 mins, 1.0ml was taken into a test tube to estimate the amount of phosphate after dialysis.

Estimation of inorganic phosphate:

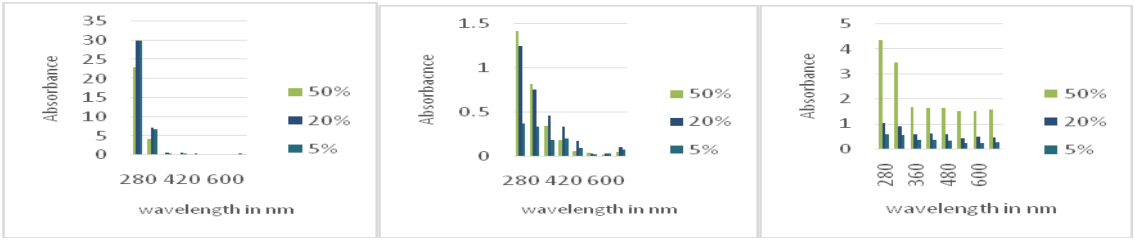
This estimation was carried out by Fiske Subbarow method [8].

RESULTS AND DISCUSSION

Organic extractions of samples:

Organic solvents are carbon based solvents that are used to dissolve other substances. They are categorised based on the molecular structure (ex: hexane is an aliphatic solvent). Solvents like acetone are used for extraction as they have the capacity to isolate compounds like phenolics, anthocyanins etc. Methanol as a solvent is used to isolate polar compound like alkaloids, lignins, terpinoids etc. Ethanol and methanol are used to elute bioactive compounds along with a few non polar compounds [9].

The organic solvents- acetone, methanol and ethanol were used in the extraction of samples- *Citrulluslanatus*seeds, *Linumustatissimum*seeds, *Helianthus annus*seeds, *Momordicacharantia, Schediumedule* and *Musaparadisiaca* stem.



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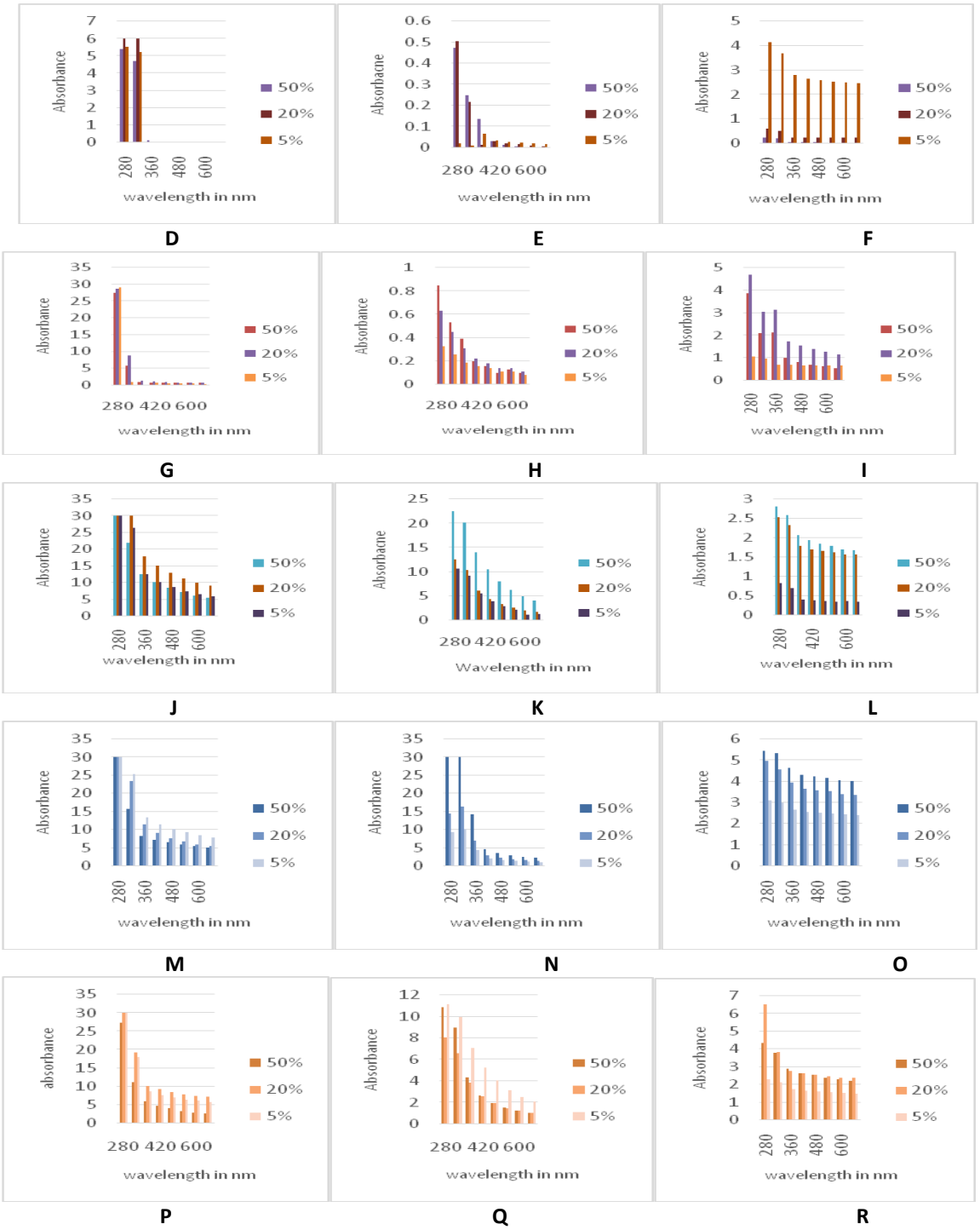

Figure 1: Showing absorbance of organic extractions for the samples taken in acetone, methanol and ethanol. A-Acetone extraction of *Momordicacharantia*, B- Methanol extraction of*Momordicacharantia*, C-Ethanol extarction of*Momordicacharantia*, D- Acetone extraction of *Musa paradisiaca* stem, E- Methenol extraction of *Musa paradisiaca* stem, F-Ethanol extraction of *Musa paradisiaca* stem, G-Acetone extraction of*Schediumedule*, H-Methanol extraction of*Schediumedule*, I-Ethanol extraction*Schediumedule*, J-Acetone extraction of *Citrulluslanatus*seeds, K-Methonal extraction of *Citrulluslanatus*seeds, L- Ethanol extraction of *Citrulluslanatus*seeds, M-Acetone extraction of *Helianthus annus*seeds, N-Methanol extraction of *Helianthus annus*seeds, O-Ethanol extraction of *Helianthus annus*seeds, P-Acetone extraction of *Linumustatissimum*seeds , Q-Methanol extraction of *Linumustatissimum*seeds, R-Ethanol extraction of *Linumustatissimum*seeds.

Most of the samples have given a high peak at 280nm. *Musaparadisiaca* stem has low absorbance at 280nm for its 50% ethanol extract. Peaks observed at ranges beyond 280 – 300nm can be due to the presence of phytochemicals. Almost all samples have shown high peaks in the acetone extraction of the samples. High

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peaks at all ranges of the ethanolic extracts of *Helianthus annus*seeds suggests that they are rich in a lot of phytochemicals (phenolics , flavonoids (Pinocembrin, pinobank-sin, gal-angin. Quercetin, etc) as well as proteins.

Buffer extractions of samples:

Buffer solvents are used mainly to maintain the integrity of the molecules, the buffer considered i.e., Phosphate and bicarbonate buffers are majorly involved in the acid -base balance of the human system. Phosphate buffer is used in the formation of urine in the kidney while bicarbonate buffer is involved in the homeostasis of the gastrointestinal tract [10].

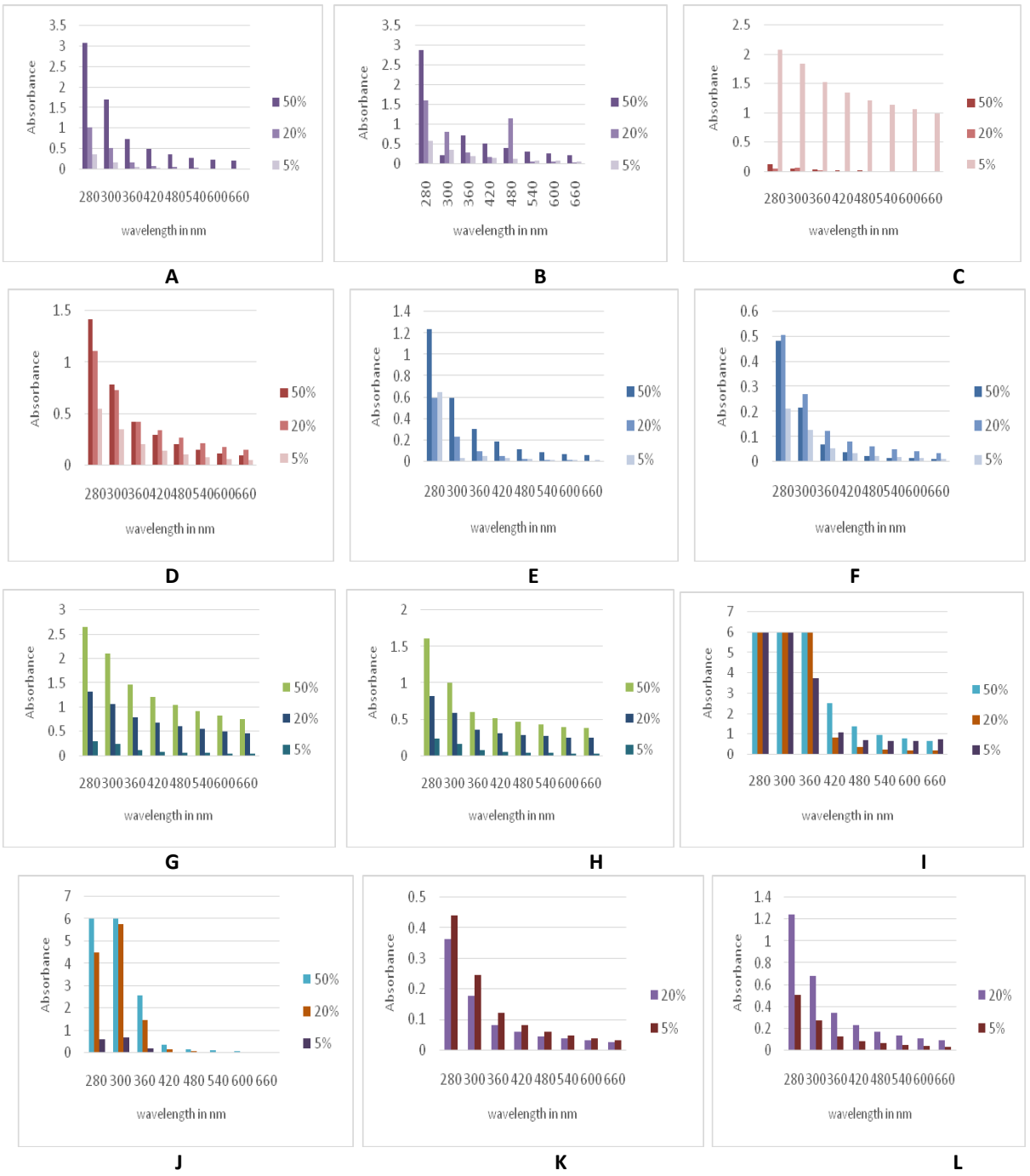

Figure 2: Showing Bicarbonate and Phosphate buffer extractions of the sample taken. A-Bicarbonate buffer extraction of *Momordicacharantia*, B-Phosphate buffer extraction of *Momordicacharantia*, C-Bicarbonate buffer extraction of *Musa paradisica*, D-Phosphate buffer extraction of *Musa paradisica*, E-Bicarbonate buffer extraction of *Schediumedule*, F-

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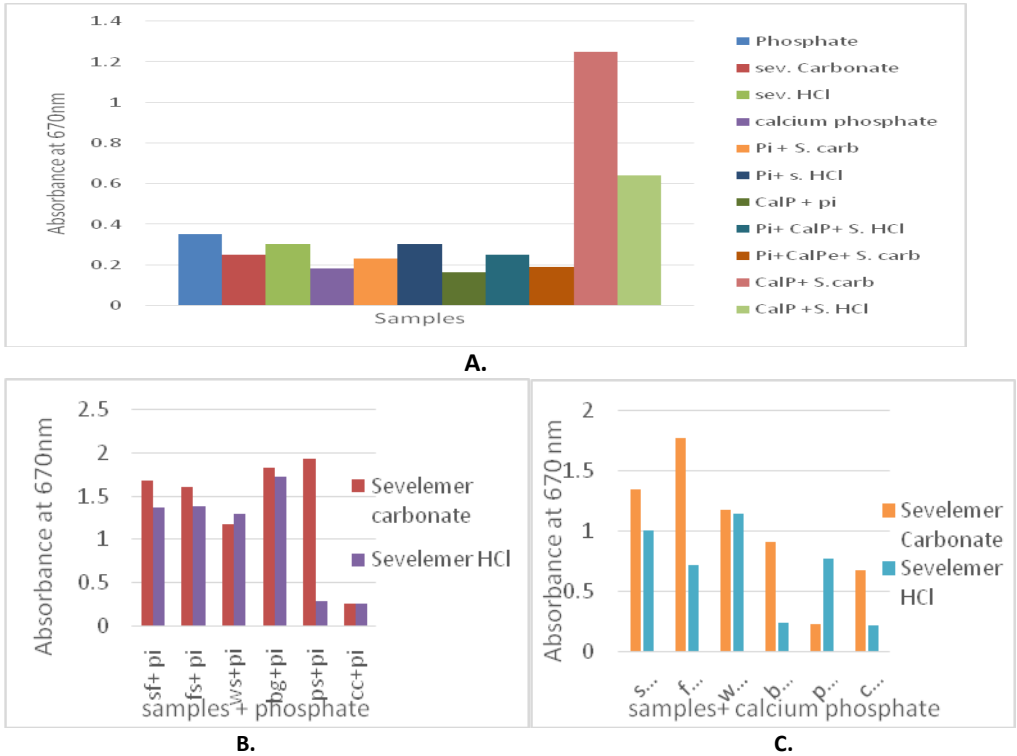
Phosphate buffer extreaction of *Schediumedule*, G-Bicarbonate buffer extraction of *Citrulluslanatus*seeds, H-Phosphate buffer extraction of *Citrulluslanatus*seeds, I-Bicarbonate buffer extraction of *Helianthus annus* seeds, J-Phosphate buffer extraction of *Helianthusannus*seeds, K-Bicarbonate buffer extraction *Linumustatissimum* seeds, L-Phosphate buffer extraction of *Linumustatissimum* seeds.

Extraction using buffer solvents were carried out so as they mimic the solvent state of the human body and studied at which concentration and buffer system the protein extraction of the plant and seed samples taken is maximum. Every sample resulted in a gradual decrease in the absorbance value from 280 to 660nm. All the samples taken shows highest peak at 280nm for both the buffer system. But for further studies Phosphate buffer samples were considered since it maintains the physiological status [10].

Phosphate estimation of dialysed samples:



Note: sf-*Helianthus annus*, fs-*Linumustatissimum*, ws-*Citrulluslanatus*, bg-*Momordica charantia*, ps-*Musa paradisiaca,* cc-*Schedium edule.*

Figure 3: A- Standard graph for Fiske-Subbarow's inorganic phosphate estimation. B- Graph showing phosphate estimation of dialysed samples obtained from plant extracts along with phosphate solution separately with the 2 drugs. C-Graph showing phosphate estimation of dialysed samples obtained from plant extracts along with calcium phosphate separately with the 2 drugs.

Dialysis carried out as a lab technique follows the same principle as that of dialysis performed for medical conditions. It is a process that aids the movement of molecules in a solution through a semi permeable membrane. It is used to remove the unwanted small molecule from macromolecules. *Sus domesticus*'s intestine was used as membrane to estimate the phosphate from the eluent. Approximately small intestine absorbs 60- 70% of phosphate. Serum phosphate does not affect the intestine uptake but influences its absorption in kidney.

The standard graph indicated highest phosphate value for the combination of Calcium phosphate and Sevelemer carbonate but the same combination was then estimated with phosphate which resulted in marginally low phosphate value indicating that presence of both phosphate and calcium phosphate reduces the efficacy of the drug. A similar result was observed with sevelamer hydrochloride as well.

Sevelemer carbonate indicated high phosphate value with plantain stem (*Musa paradisiacal)* as well as bitter gourd (*Momordica charantia)* and least with squash (*Schedium edule*)

when estimated with the drug and phopshate. The phosphate value of the same samples for sevelamer hydrochloride is low when compared to sevelamer carbonate indicating that carbonate form of the drug is more efficient.

The graph obtained along with calcium phosphate gave the highest phosphate value with flax seeds and least with plantain stem. This might be due to some kind of interaction of the sample components with calcium phosphate that reduces its efficacy. In this case as well the hydrochloride form of the dug is less efficient than carbonate.

Absorption is also dependent on the form of the drug (tablet, powder etc.). It is known from the earlier studies that the powder form of carbonate and tablet form of sevelamer hydrochloride are equivalent in their absorption capacity [11]. Current study is the difference obtained when the 2 forms of the drug is considered in the tablet version.

SUMMARY

In the present investigation, several parameters were taken to study the drug, its effect on removal of phosphate by inducing hyperphosptemia conditions, its effect in combination with the selected samples.

Organic and buffer solvent extractions were carried out using acetone, methanol, ethanol, bicarbonate buffer and phosphate buffer on *Citrullus lanatus* (watermelon) seeds, *Helianthus annus* (sunflower) seeds, *Linum ustatissimum* (flax) seeds, *Momordica charantia* (bitter gourd)*, Musa paradisiaca* (plantain)stem *and Schedium edule* (squash). The extractions were carried out to identify the presence of proteins and other phytochemicals in the sample.

Dialysis of the drug, drug- induced with phosphate and calcium phosphate, along with other samples were investigated in-vitro and the eluents obtain after was analysed for the phosphate values using Fiske-Subbarow method. It was observed that phosphate value varied for different samples and also depends on the presence of phosphate and calcium phosphate. It leads to a conclusion that squash affects the efficiency of the drug and hence should not be recommended for patients suffering from CKD and hyperphosphatemia.

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