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BI-PHASE EXTRACTION OF PARTIALLY PURIFIED SOLANUM TUBEROSUM, COLOCASIA ESCULENTA, PUNICA GRANATUM AND PHASEOLUS VULGARIS ALKALINE PHOSPAHTASES

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ABSTRACT

Alkaline phosphatase was isolated and purified from Solanum tuberosum (potato tubers) by a streamline method without the use of proteolytic and lipolytic enzymes and butanol. The study features are the use of a buffer solutions, ammonium sulphate precipitation at low temperature to remove impurity and the use of polyethylene glycol solution with potassium phosphate solution to concentrate the partially purified Solanum tuberosum , Colocasia esculenta (Taro), Punica granatum (pomegranate) and Phaseolus vulgaris (French beans) alkaline phosphatase by bi-phase extraction method. Enzyme activity of the extracted & purified Solanum tuberosum, Colocasia esculenta (Taro), Punica granatum (pomegranate) and Phaseolus vulgaris (French beans) alkaline phosphatase was done by para-nitro-phenyl-phosphate method (p-NPP method) and absorbance was taken at 410nm.

Keywords:

Solanum tuberosum (Potato tubers*; Colocasia esculenta* (Taro); *Punica granatum* (pomegranate); *Phaseolus vulgaris* (French beans); alkaline phosphatase; para-nitro-phenyl-phosphate method; Polyethylene glycol; bi-phase extraction.

Number of Tables : 1 **Number of Refernces :** 22

INTRODUCTION

Alkaline phosphatases have been traditionally classified as being alkaline, due to its optimum PH activity, above PH 7.0 (Barret – Lannard et al., 1982; Sharma et al., 2004). Alkaline phosphatase (EC 3.1.3.1) are enzymes that catalyze the removal of inorganic phosphate (orthophosphate) from organic phosphate esters, in alkaline media (Panara et al., 1990; Vincent et al., 1992; Asmar et al., 1995).These phosphatases are ubiquitous in plants, animals and microorganisms (Lee, 1988; Duff et al., 1994; Jeong et al., 2003). Alkaline phosphatases in plants play a major role in the supply and metabolism of inorganic phosphate for the maintenance of cellular metabolism (Tabaldi et al., 2007; Mishra and Dubey, 2008). Alkaline phosphatase has also been reported to be involved in the breakdown and mobilization of starch and sucrose, for the biosynthesis of essential oil in lemongrass Cymbopogon flexuosus Steud) Wats (Ganjewala et al., 2010). Mishra and Dubey (2008) also reported the inhibitory effect of Arsenite (As_2O_3) on the activities of alkaline phosphatases in rice (*Oryza sativa L.*) seedlings, which resulted in a decline in the level of the phosphate pool. Inorganic phosphate plays a vital functional role in energy transfer and metabolic regulation and is also an important structural constituent of many biomolecules. Consequently, inorganic phosphate metabolism is of critical importance in plant developmental processes (Julie et al., 2000; Bozzo et al., 2002). Dry-matter yield, phosphate uptake, acid and alkaline phosphatase activity and microbial population were increased in all the phosphate treatments. Organic Phosphate enhanced alkaline phosphatase activity. Lecithin increased fungal, and phytin bacterial growth. There was no alkaline phosphatase activity in the asepticallly grown clover root exudates. Phosphatase released in aseptic culture after 4 weeks of clover growth was able to efficiently hydrolyze sodium glycerophosphate, lecithin and phytin. The amount of organic phosphate hydrolyzed in this and in the soil experiment surpassed plant uptake by 20 times (Tarafdar J.C. and Claassen N., 1998). Plant seeds are enriched in thermostable alkaline phosphatase such as *Vigna unguiculata, Cajanus indicus, Arachis hypogaea* (Kumar P. V. et al, 2010). This study was to identify, isolate and quantify the level of alkaline phophatase in *Solanum tuberosum* (potato tubers), *Colocasia esculenta* (taro), *Punica granatum* (pomegranate) and *Phaseolus vulgaris* (French beans).

MATERIALS AND METHODS

Preparation of crude enzyme extract of *Solanum tuberosum* (potato tubers): Potato tubers was collected from a local area and washed with tap water followed by double distilled water with two times. The skin was peeled off and 20 gms of *Solanum tuberosum* (potato tubers) was weighed. After weighing, *Solanum tuberosum* (potato tubers) was sliced out into very small pieces and washed again two times with distilled water. And after washing, these small pieces of *Solanum tuberosum* (potato tubers) were

crushed in pestle mortar in 8-10 ml of potassium phosphate buffer (pH 7). All preparations of crude extract of *Solanum tuberosum* (potato tubers) was carried out in cold conditions (0-4°C). After crushing, crude extract was centrifuged at 12000 rpm for 15 minutes. Supernatant was collected and stored at 4°C.

Preparation of crude enzyme extract of *Colocasia esculenta* (taro): Taro was collected from a local area and washed with tap water followed by double distilled water with two times. Then, these washed *Colocasia esculenta* (taro) were soaked in double distilled water for 2-5 hrs. After soaking, the skin was peeled off and 20-30 gms of *Colocasia esculenta* (taro) was weighed. Rest of the procedure was followed as used for preparation of crude enzyme extract of *Solanum tuberosum* (Potato tubers).

Preparation of crude enzyme extract of *Punica granatum* (pomegranate): Pomegranate was collected from a local area and peeled off. And, the ripened seed of this fruits were washed with tap water followed by double distilled water with two times. Then, 20 gms of these washed ripened seed of *Punica granatum* (pomegranate) was weighed. Rest of the procedure was followed as used for preparation of crude enzyme extract of *Solanum tuberosum* (Potato tubers).

Preparation of crude enzyme extract of *Phaseolus vulgaris* (French beans): *Phaseolus vulgaris* (french beans) was collected from a local area and washed with tap water followed by double distilled water with two times. Then, 20 gms of *Phaseolus vulgaris* (french beans) was weighed. Rest of the procedure was followed as used for preparation of crude enzyme extract of *Solanum tuberosum* (Potato tubers).

Ammonium sulphate precipitation in crude extract of *Solanum tuberosum*: Crude extract of *Solanum tuberosum, Colocasia esculenta, Punica granatum* and *Phaseolus vulgaris* alkaline phosphatase was placed in ice bath and 80% ammonium sulphate (gm of ammonium sulphate per 100 ml of crude enzyme extract) was added in small proportions (two pinches) till then the saturation point reached. Continuous stirring was done with each small addition (two pinches) of ammonium sulphate salt. Medium was continuously stirred for 30 minutes after complete addition of ammonium sulphate or complete saturation. Then, the medium was kept at least for one hour for complete precipitation. After one hour, medium containing protein precipitate was centrifuged in cold centrifuge at 10,000 rpm for 30 minutes. Supernatant was discarded. Pellet was collected carefully with the help of pipette and dissolved in 2-5ml of 0.05M 5 ml of sodium acetate buffer having pH 7.

Bi-phase extraction of ammonium sulphate precipitated *Solanum tuberosum* alkaline phosphatase: 20ml of 30.7% of K_2HPO_4 (7 parts) and KH_2PO_4 (3 parts) solution was taken in test tube and add 2ml 5% Polyethylene glycol solution. Drop wise addition of polyethylene glycol was done and vortexed after every addition. Now, 1ml of ammonium sulphate precipitated *solanum tuberosum* alkaline phosphatase was added

and vortex for 15-20 minutes. After voetexing, a dark brown coloured layer was observed which contained bi-phase extracted alkaline phosphatase and it was pipetted out carefully.

Bi-phase phase extraction of ammonium sulphate precipitated *Colocasia esculenta* alkaline phosphatase: 20ml of 30% of K_2HPO_4 (7 parts) and KH_2PO_4 (3 parts) solution was taken in test tube and add 2ml 5% Polyethylene glycol solution. Rest of the procedure was followed as done for bi-phase extraction of ammonium sulphate precipitated *Solanum tuberosum* (potato tubers).

Bi-phase extraction of ammonium sulphate precipitated *Punica granatum* alkaline phosphatase: 20ml of 30% of K_2HPO_4 (7 parts) and KH_2PO_4 (3 parts) solution was taken in test tube and add 2ml 5% Polyethylene glycol solution. Rest of the procedure was followed as done for bi-phase extraction of ammonium sulphate precipitated *Solanum tuberosum* (potato tubers).

Bi-phase extraction of ammonium sulphate precipitated *Phaseolus vulgaris* alkaline phosphatase: 20ml of 31% of K_2HPO_4 (7 parts) and KH_2PO_4 (3 parts) solution was taken in test tube and add 2ml 5% Polyethylene glycol solution. Rest of the procedure was followed as done for bi-phase extraction of ammonium sulphate precipitated *Solanum tuberosum* (potato tubers).

Activity test of crude extract, ammonium sulphate precipitated and bi-phase extracted *Solanum tuberosum*, *Colocasia esculenta, Punica granatum* (pomegranate) and *Phaseolus vulgaris* (French beans) alkaline phosphatases by para-nitro-phenyl-phosphate (p-NPP) method: 5 ml glycine NaOH (pH 10.5) buffer was taken in test tubes and 0.1ml $MgCl_2$, 0.1 ml of para-nitro-phenyl phosphate (p-NPP) was added. Add 0.5 ml of crude extract, ammonium sulphate precipitated and bi-phase phase extracted *Solanum tuberosum* (potato tubers), *Colocasia esculenta* (taro), *Punica granatum* (pomegranate) and *Phaseolus vulgaris* (French beans) alkaline phosphatases were added to in the reaction mixture. Then, the test solution was incubated at 37°C for 10 minutes. After incubation, 2ml 0.085N NaOH was added in each test tube to stop the reaction and absorbance is taken at 410nm. The standard curve of para-nitro-phenol (p-NP) was prepared with above given test method by replacing the test sample with para-nitro-phenol solution in the concentrations ranging from 0.5µ mol/ml to 4.5 5µ mol/ml Vs absorbance taken at 410nm. The absorbance of test samples was extrapolated with the concentrations of prepared standard curve of para-nitro-phenol Vs absorbance at 410nm.

RESULTS AND DISCUSSION:

Enzyme activity of all the three types of *Solanum tuberosum, Colocasia esculenta*, *Punica granatum* and *Phaseolus vulgaris* alkaline phosphatase such as crude extract, ammonium sulphate precipitated extract and bi-phase extracted extract was given in table 1. Previously, no work was done on the

extraction and purification of alkaline phosphatase from *Solanum tuberosum* (potato tubers), *Colocasia esculenta* (taro), *Punica granatum* (pomegranate) and *Phaseolus vulgaris* (French beans). Thus, from our results, it was confirmed that *Solanum tuberosum* (potato tubres) had alkaline phosphatase activity and the % of purification was obtained in the range of 70-75% from ammonium sulphate precipitation and Polyethylene glycol bi-phase extraction in *Solanum tuberosum* (Potato tubers). *Solanum tuberosum* (potato tubers) is a type of heptoprotective plants which has activity of alkaline phosphatase and result was comparable to *Solanum nigram* which also has alkaline phosphatase activity as postulated by Mohamed Saleem et al, 2010. On other hand, *Colocasia esculenta* (taro) which is a underground root tubers has alkaline phosphatase activity which is also a type of root tuber buried in the soil. As well as the % of purification was obtained in the range of 72% from ammonium sulphate precipitation and Polyethylene glycol bi-phase extraction. Thus, these plant roots of various plants such as clover, barley, oats and wheat have alkaline phosphatase which was confirmed by Tarafdar J.C. & Claassen N., 1998 and hence, our result was comparable with this results that shoot (shoot tubers) and root rubbers (taro). And, *Punica granatum* (pomegranate) is a type of fruit which has also the alkaline phosphatase activity (Table 1) by our finding. This result was comparable to the reports which were collected in the review on hepatoprotective herbal plants such as *Solanum nigram, Flaceurtia indica, Silybum marianum, Chamomile capitula, Cocina grandis, Annona squamosa, Wedelia calendulacea, Prostecha michuacana, Ficus caica, Lepidium sativm, Aegle marmelos, Sargassaum polycystum and Orthosiphnon stamineus.* Hence, these are all herbal/medicinal plants, have alkaline phosphatase enzyme and categorized in the hepatoprotective herbal plants which help in the recovery of damaged liver/injured liver during pathological conditions as postulated by Mohamed Saleem et al, 2010. The % of purification was obtained in the range of 71-75% from ammonium sulphate precipitation and Polyethylene glycol aqueous two phase extraction. Our results were also comparable with the finding of Kumar P.V. et al, 2010 who confirmed the presence of thermostable alkaline phosphatase in various plant seeds such as yellow pea *(Pisum sativum)* Ladies finger (*Abelmoschus esculentus*), Chick pea (*Cicer arietinum*), Groundnut (*Arachis hypogaea*), Soya bean Bitter gourd (*Momordica charantia*), Black eyed bean (*Vigna unguiculata*), Red gram (*Cajanus indicus*), Green gram (*Vigna radiates*) Black gram (*Phaseolus mungo*).

Table 1: Enzyme Activity of crude extract, ammonium sulphate precipitated and bi-phase extracted *Solanum tuberosum, Colocasia esculenta, Punica granatum* and *Phaseolus vulgaris* alkaline phosphatase.

Type of extracts	*Solanum tuberosum	*Colocasia esculenta	*Punica granatum	*Phaseolus vulgaris
Crude	82	20	20	67.2
Ammonium sulphate precipitated	140	76	72	118
Bi-phase extracted	144	72	72	115

*Enzyme activity (µ mol/ml) by p-nitro-phenyl-phosphate (p-NPP) method at 410nm

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