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L-ASPARAGINASE FROM *PHYLLANTHUS EMBLICA* (AMLA): A NOVEL SOURCE

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ABSTRACT: The biologically active compounds in plants reckoned their application in medicines from ancient times. Among the biologically active compounds enzymes have evolved as a potential therapeutic agent. L-asparaginase a therapeutic protein is extensively employed in medical sector to diagnose and treat leukemia. Bacteria, fungi, yeast and plants have been identified as potential sources of L-asparaginase. The side effects associated with microbial L-asparaginase restricted their utilization in treatment of leukemia and bought medicinal plants as source into focus. In the present study, different species of *Phyllanthus* genera have been screened for presence of L-asparaginase in different seasons. The leaves of *Phyllanthus emblica* with highest enzyme activity (20.3 IU/ml) and specific activity (5.2 IU/mg) emerged out as potential source for L-asparaginase production. *Phyllanthus amarus* and *Phyllanthus niruri* also contained appreciable amount of L-asparaginase. This is the first report of identifying presence of L-asparaginase in *Phyllanthus* genera. L-asparaginase from *Phyllanthus emblica* was further purified to homogeneity and the molecular mass of enzyme was found to approximately 85kDa. The yield of purified enzyme was 63.54% with 8.5 fold increase in specific activity. Kinetic parameters, K_m and V_{max} of enzyme, were found to be 3.83 mM and 292.141mM/min, respectively.

INTRODUCTION: Among the biologically active compounds present naturally, enzymes due to their high affinity, specificity and catalytic efficiency has evolved as potential therapeutic agent and has found application in food and textile industries. L-asparaginase from amidohydrolase family catalyzes the breakdown of L-asparagine to L-aspartic acid and ammonia. This action mechanism of L-asparaginase unwrapped various applications of enzyme in food and medical sector.

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L-asparaginase enzyme has evolved as anti-leukemic and anti lymphoma agent and reckoned as the largest group of therapeutic enzyme [1]. Unlike normal cells tumor cells rely on asparagine from body fluids for their growth. The asparaginase when injected as anti leukemic agent reduces the free circulating asparagine, leading to starvation of asparagine in susceptible tumor cells [2, 3].

L-asparaginase is also used in food processing industry to avoid the formation of acrylamide (tumor causing agent) during production of starchy food products and as quality assurance parameter for fruit juices [4] and vegetables [5]. L-asparaginase is widely distributed among bacteria, yeast, fungi, actinomycete, plants and rodent serum. Microorganisms such as bacteria, fungi, yeast, actinomycetes and algae have been identified as

proficient sources of L-asparaginase. Commercially available L-asparaginase from *Escherichia coli* and *Erwinia carotovora* has been extensively acquired as a chemotherapeutic agent in treatment of leukemia [6]. The L-asparaginase from microbes has low specificity to asparagine leading to sensitivity and toxic side effects in patients treated with enzyme. The side effects associated with microbial L-asparaginase turned the focus of research for screening of other source for L-asparaginase production.

L-asparaginase has been extracted from various plant species *Lupinus leuteus, Dolichos lablab* seedlings [7], *Capsicum annum, Tamarindus indica* [8], *Pisum sativum* [9], *Lupinus arboreus, L. angustiplius* [10] and in the root soil of *Pinus pinaster* and *Pinus radiate* [11]. L-asparaginase encoding cDNA sequence has been isolated from soybean leaves [12]. *Withania somnifera* has been reported as a potential source of L-asparaginase and its different cytotypes were compared [13, 14]. L-asparaginase was also found in Citrus lemon [15] and *Solanum nigrum* [16]. Plants have emerged as competent source of enzymes as plant enzymes are easy to handle, have fewer chances of pathogenicity and can be used crude to develop drug formulations which saves cost and time. Also according to literature reports the utilization of L-asparaginase from plants for treatment of acute lymphoblastic leukemia has been studied less [17]. Different species of *Phyllanthus* genera have been utilized as traditional medicinal value plants from ancient time and have been involved in treatment of common ailments like diabetes, ulcers and urinary treatments [18]. The current work is focused towards screening of different species of *Phyllanthus* genera for presence of L-asparaginase, followed by purification and characterization of isolated L-asparaginase which could act as potential anti tumor agent with negligible side effects due to immunological responses.

MATERIALS AND METHODS:

Plant Material: Different plant parts of different species of *Phyllanthus* genera were collected in different seasons in sterile polythene bags **Table 1**. The plants are cultivated and well documented by the Department of Botany, Punjab Agriculture University, Punjab.

TABLE 1: SEASON AND PHARMACOLOGICAL RELEVANCE OF SCREENED SPECIES OF *PHYLLANTHUS* GENERA

S. no.	Plants	Flowering / Fruiting Season	Pharmacological Significance	Reference
1	*Phyllanthus emblica*	December –March July-August	Acts as antioxidant, immune modulatory, antipyretic, analgesic, cytoprotective, anti ulcer, anti microbial, immune modulatory, anti inflammatory and gastroprotective. Plays active role in treatment of peptic ulcer, dyspepsia, jaundice, pradara, diabetes	19, 20
2	*Phyllanthus amarus*	July-August	To treat stomach, genitourinary system, liver, kidney, and spleen problems. To treat jaundice, gastropathy, diarrhoea, dysentery, fevers, menorrhagia, scabies, genital infections, ulcers, gonorrhea and syphilis and skin disease, acts as antidiabetic, ant-malarial, antimicrobial and anti mutagenic agent	21, 22
3	*Phyllanthus niruri*	July -August	Acts as antioxidant, antimicrobial, anti-inflammatory and anticancer agent. It is used to lower down the blood sugar, prevents ulcers and kidney stones. It is used in treatment of hepatitis B and improves liver health	23, 24
4	*Phyllanthus urinari*	July-August	Acts as anticancer, anti-inflammatory, anti bacterial and antioxidant agent. Involved in treatment of jaundice, diabetes ulcers, and hypertension	25, 26

Extraction of L-asparaginase: Different parts of selected species of *Phyllanthus* genera were collected and washed with distilled water. The parts were crushed and homogenized with 0.1M KCl buffer (pH 8.6). It was then centrifuged at 8000 rpm for 20 min at 4 ºC and filtered. The supernatant thus obtained was taken as crude enzyme [8].

Enzyme Assay: Nessler's method based on estimation of ammonia released on breakdown of asparagine by the enzyme was adopted for L-asparaginase assay [27]. The reaction between Nessler's reagent (K_2HgI_4) and ammonia leads to production of pale yellow color. The color intensity is directly proportional to the amount of ammonia present. The standard graph of ammonium sulphate was plotted. Further the enzyme activity of crude enzyme was determined by Nessler's method and the intensity of pale yellow color was determined

by taking absorbance at 480 nm. The micromole of ammonia produced was determined from ammonium chloride standard curve.

Protein Estimation: In order to determine the specific enzyme activity the crude enzyme was subjected to protein estimation by Folin-Lowry's method with bovine serum albumin (BSA) as standard [28].

Purification of L-asparaginase: L-asparaginase enzyme purification was carried out sequentially according to steps proposed by Scopes [29].

Ammonium Sulphate Precipitation: The crude enzyme was precipitated out by varying the ammonium sulphate saturation from 20% to 100% at 4 °C under mild stirring conditions. The sample was kept for 12 h at 4 °C without stirring. When the precipitation occurred, the sample was centrifuged for 20 min (5000 rpm at 4 °C) and pellet was dissolved in phosphate buffer saline.

Dialysis: The precipitate obtained after ammonium sulphate precipitation was poured in activated dialysis membrane tube and placed in buffer solution (0.01M Na borate buffer). Enzyme activity and protein content of the dissolved pellet and supernatant was quantified after the dialysis.

Q-Sepharose Strong Anion Exchange Chromatography: The dialyzed sample was loaded onto Q Sepharose chromatographic column (1.2 × 8.2 cm) pre-equilibrated with 0.01M sodium borate buffer (pH 8.6) at a flow rate of 1 ml min^{-1}. The column was washed with 0.01M sodium borate buffer (pH 8.6) to remove the unbound proteins.

Further the elution was carried out by raising the NaCl salt gradient from 0.1M - 0.6M and 1.5 ml fractions were collected at flow rate of 1 mL/min. The fractions were evaluated for enzyme activity and protein concentration.

Molecular Weight Determination: Fractions with highest specific activity along with protein marker were consequently loaded along with loading sample into polyacryl amide gel (10%) as supporting medium to determine the molecular weight and purity of L-asparaginase. Electrophoresis was carried out at 100V. The gel was washed with deionised water and thereafter stained with bromophenol blue and visualized with comassie brilliant blue.

Kinetic Characterization of L-asparaginase: Further L-asparaginase kinetics was studied to determine the effect of experimental parameters like pH, temperature and substrate concentration on the rate of reaction. In order to determine the effect of pH on enzyme activity, the crude enzyme was incubated in 0.01 M sodium borate buffer of different pH range (5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5). The reaction mixture was incubated at different temperature range (4-80 ºC) for 15 min in 0.01 M sodium borate buffer (pH 8.6) to determine the effect of temperature on reaction rate. The determination of effect of substrate concentration was achieved by Lineweaver-Burk's plot [30] using different L-asparagine concentrations (1.0 to 10.0 mM).

RESULTS AND DISCUSSION:

Potential Plant Source of L-Asparaginase: Different plant parts of selected species of *Phyllanthus* genus in different seasons were screened for presence of L-asparaginase **Table 2**. Among all plants, maximum L-asparaginase activity was observed in *Phyllanthus emblica* (HS16497) leaves during the non fruiting season. Appreciable amount of L-asparaginase was also observed in *Phyllanthus amarus*, *Phyllanthus niruri* and *Phyllanthus urinari.* In all the plants, leaves were identified as potential source for extraction of enzyme in comparison to other plant parts and maximum enzyme activity in leaves was observed during the non fruiting season. Therefore, the results inferred that maximum concentration of L-asparaginase is found in plants at the time of synthesis of new proteins *i.e.* before the fruiting season. Similar results with high L-asparaginase activity in different medicinal plants before fruiting season has been reported [31].

Protein Estimation: The leaves of screened plants showed maximum enzyme activity. The protein estimation and specific activity of the leaves of screened medicinal plants was estimated. The leaves of *Phyllanthus emblica* showed maximum specific activity of 5.2 IU/mg for L-asparaginase enzyme. Considerable enzyme specific activity was observed in *Phyllanthus amarus* and *Phyllanthus niruri* **Table 3**.

TABLE 2: COMPARATIVE SCREENING ACCOUNT OF SELECTED SPECIES OF *PHYLLANTHUS* GENERA FOR L-ASPARAGINASE PRODUCTION

S. no.	Plants	Parts	Season	Enzyme activity (IU/ml)
1	*Phyllanthus emblica*	Leaves	Non-fruiting Season	20.3 ± 0.42
			Fruiting Season	18.5 ± 0.51
		Stem	Non-fruiting Season	9.2 ± 0.21
		Fruit	Fruiting Season	11.5 ± 0.26
2	*Phyllanthus amarus*	Leaves	Non fruiting Season	18.1 ± 0.55
			Fruiting Season	11.1 ± 0.51
		Stem	Non-fruiting Season	8.9 ± 0.21
		Fruit	Fruiting Season	7.7 ± 0.17
3	*Phyllanthus niruri*	Leaves	Non-fruiting Season	17.5 ± 0.51
			Fruiting Season	11.8 ± 0.51
		Stem	Non-fruiting Season	8.1 ± 0.36
		Fruit	Fruiting Season	7.4 ± 0.22
4	*Phyllanthus urinary*	Leaves	Non-flowering Season	16.4 ± 0.33
			Flowering Season	10.8 ± 0.32
		Stem	Non-flowering Season	5.7 ± 0.15
		Flowers	Flowering Season	3.9 ± 0.07

TABLE 3: PROTEIN CONTENT AND SPECIFIC ACTIVITY OF L-ASPARAGINASE ISOLATED FROM DIFFERENT SPECIES OF *PHYLLANTHUS* GENERA

S. no.	Plants (leaves)	Enzyme activity (Units/ml)	Protein content (mg/ml)	Specific activity (Units/mg)
1	*Phyllanthus emblica*	20.3 ± 0.42	3.9172 ± 0.12	5.2 ± 0.21
2	*Phyllanthus amarus*	18.1 ± 0.55	4.154 ± 0.22	4.1 ± 0.3
3	*Phyllanthus niruri*	17.5 ± 0.51	4.44 ± 0.23	3.9 ± 0.22
4	*Phyllanthus urinari*	16.4 ± 0.33	4.693 ± 0.25	3.4 ± 0.07

Purification: L-asparaginase from leaves of *Phyllanthus emblica* with maximum specific activity was further purified by ammonium sulphate precipitation and Q-sepharose anion exchange chromatography. The specific activity of the crude extract improved from 5.2 units/mg to 16.6 units/mg after purification. 40-60% saturation of ammonium sulphate fractionation showed 3.19 fold purification of enzyme with 73% recovery.

TABLE 4: PURIFICATION SCHEME OF L-ASPARAGINASE FROM *PHYLLANTHUS EMBLICA* LEAVES

Sample	Enzyme activity (IU/ml)	Protein (mg/ml)	Specific activity (IU/mg)	Purification Fold	Yield / Recovery %
Crude	20.3 ± 0.42	3.9 ± 0.12	5.2 ± 0.21	-----	100
40-60%	15 ± 0.03	0.9 ± 0.3	16.6 ± 0.19	3.19	73
Ion Exchange	12.9 ± 0.009	0.29 ± 0.07	44.4 ± 0.04	8.5	63.54

Further, the extract was pooled through Q-sepharose anion exchange chromatography. Activity was increased by 8.5 fold when eluted with 0.5 M NaCl solution with 63.54% recovery. The results for purification of L-asparaginase are summarized in **Table 4**. Comparative purification results have been reported in plants like green chilli [8], pea [32] and *Withania somnifera* [33].

Single band in the electrophoresis study further confirmed the purity achieved through purification steps. Approximate molecular weight of purified enzyme was found to be 85kDa, as determined by comparing it against protein marker ladder on gel **Fig. 1**. The findings are in comparison with L-apsarginase isolated from *Withania somnifera* with molecular weight of 72 KDa [13] and from *Phaseolus vulgaris* seeds of molecular weight 79 KDa [34].

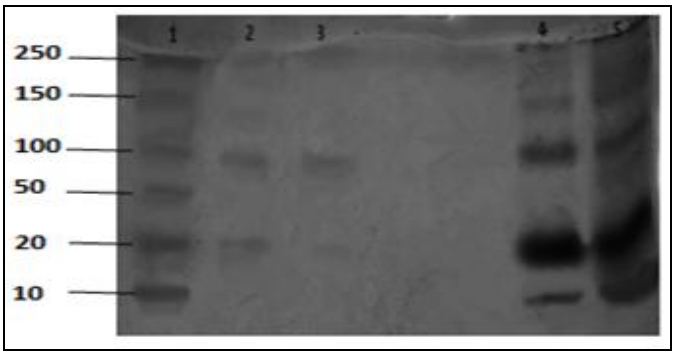

FIG. 1: MOLECULAR WEIGHT DETERMINATION OF L-ASPARAGINASE FORM *PHYLLANTHUS EMBLICA* LEAVES. (1) Broad range (10-250 kD) Protein marker (2) Ammonium sulphate precipitation (3) Q-Sepharose anion exchange chromatography (4) and (5) Crude enzyme

Kinetic Characterization of L-asparaginase: Kinetic parameters of purified L-asparaginase from *Phyllanthus emblica* leaves was determined by varying the pH, temperature and substrate concentration. Using Lineweaver-Burk plots **Fig. 2** K_m and V_{max} for enzyme were found to be 3.83 mM and 292.141mM/min respectively. The K_m obtained is found to be comparable to L-asparaginase purified from green chilli (3.3 mM) [8] and lower in comparison to *L. arboreus* and *L. angustifoliusw* K_m value of 6.6 and 7.0mM respectively [35] .The K_m value was also found to be comparative with K_m value of L-asparaginase from *Escherichia coli* (3.5 mM) and *Erwinia carotovora* (7.14mM) [36, 37]. L-asparaginase from *Phaseolus vulgaris* seeds also showed higher K_m value of 6.72mM [34].

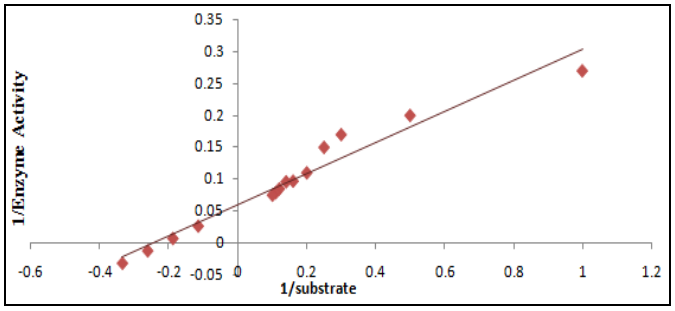

FIG. 2: LINEWEAVER–BURK PLOT FOR THE DETERMINATION OF K_M AND V_{MAX} FOR L-ASPARAGINASE FROM *PHYLLANTHUS EMBLICA*

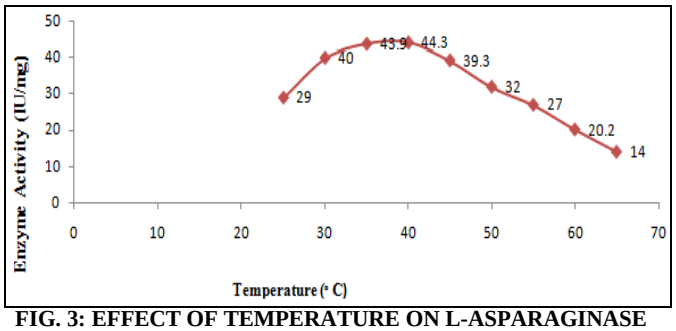

FIG. 3: EFFECT OF TEMPERATURE ON L-ASPARAGINASE ACTIVITY EXTRACTED FROM *PHYLLANTHUS EMBLICA*

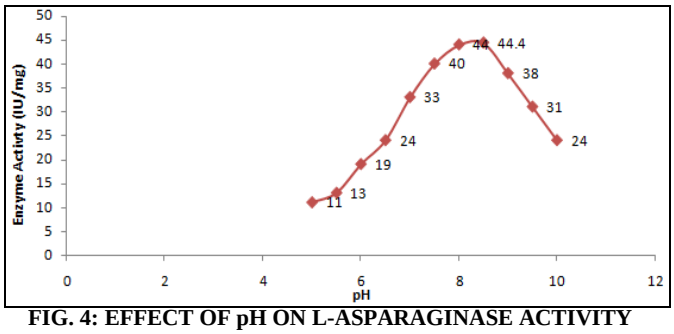

FIG. 4: EFFECT OF pH ON L-ASPARAGINASE ACTIVITY EXTRACTED FROM *PHYLLANTHUS EMBLICA*

The extract from *Phyllanthus emblica* showed maximum activity at 37ºC **Fig. 3** and 8.5 pH **Fig. 4**. The optimum pH and temperature were found to be comparative with that of L-asparaginase extracted from microorganisms like *Erwinia carotovora* [37] and *Pectobacterium carotovorum* [38]. Similar optimum pH and temperature have been reported for L-asparaginase extracted from various plants like *Vigna unguiculata* [39], *Withania somnifera* [33], *Solanum nigrum* [16] and *Phaseolus vulgaris* seeds [34].

CONCLUSION: Four species of *Phyllanthus* genera were explored for presence of L-asparaginase with highest activity in *Phyllanthus emblica*. Purified L-asparaginase from *Phyllanthus emblica* showed comparative similarities with bacterial L-asparaginase. The low K_m value of 3.83 mM and high V_{max} of 292.141mM/min illustrated high affinity of enzyme towards the substrate. Moreover, the enzyme showed stability over a range of temperature and pH. Furthermore, plant enzymes have lesser chances of pathogenicity and side effects, thus L-asparaginase extracted from *Phyllanthus emblica* could act as potential therapeutic protein and could be widely further explored for its anti leukemic activity.

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CONFLICT OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Warangkar SC, Khobragade CN, Dawane BS and Bhosale RB: Effect of dihydropyridine deravatives on the kinetic parameter of E. caratovora L-asparaginase. International Journal of Biotechnology Applications 2009; 1: 5-13.
2. Killander D, Dohlwitz A, Engstedt L, Franzen S, Gahrton G, Gullbring B, Holm G, A. Holmgren A, Hoglund S, Killander A, Lockner D, Mellstedt H, Moe PJ, Palmblad J, Reizenstein P, Skarberg KO, Swedberg B, Uden AM, Wadman B, Wide L and Ahstrom L: Hypersensitive reactions and antibody formation during L-asparaginase

treatment of children and adults with acute leukemia. Cancer 1976; 37: 220-228.
3. Patil S, Coutsouvelis J and Spencer A: Asparaginase in the management of adult acute lymphoblastic leukemia: is it used appropriately? Cancer Treatment Review 2011; 37: 202-207.
4. Babsky N, Toribio JL and Lozano JE: Influence of storage on the composition of clarified apple juice concentrate. Journal of Food Science 1986; 51: 564-567.
5. Hurst PL, Boulton G and Lill RE: Towards a freshnees test for asparagus: spear tip asparagine content is strongly related to post-harvest accumulated heat-units. Food Chemistry 1998; 61: 381-384.
6. Marlborough DI, Miller DS and Cammack KA: Comparative study on conformational stability and subunit interactions of two bacterial asparaginases. Biochimica et Biophysica Acta 1975; 386: 576-589.
7. Lees EM and Blakeney AB: The distribution of asparaginase activity in legumes, Biochimica et Biophysica Acta 1970; 215: 145-151.
8. Bano M and Sivaramakrishnan VM: Preparation and properties of L-asparaginase from green chillies (*C. annum* L.). Journal of Biosciences 1980; 2:291-297.
9. Sodek L, Lea PJ and Miflin BJ: Distribution and properties of potassium dependent asparaginase isolated from developing seeds of *Pisum sativum* and other plant. Plant Physiology 1980; 65: 22-26.
10. Borek D, Podkowinski J, Kisiel A and Jaskolski M: Isolation and characterization of cDNA encoding L-asparaginase from *Lupinus luteus*. Plant Physiology 1999; 119: 1568-1570.
11. Bell TL and Adams MA: Ecophysiology of ectomy-corrhizal fungi associated with *Pinus spp*. Plant Ecology 2004; 171: 35-52.
12. Cho CW, Lee HJ, Chung E, Kim KM, Heo JE, Kim JI, Chung J, Ma Y, Fukui K, Lee DW, Kim DH, Chung YS and Lee JH: Molecular characterization of the soybean L-asparaginase gene induced by low temperature stress. Chinese Academy of Agricultural Sciences 2007; 23: 280-286.
13. Oza VP, Trivedi SD, Parmar PP and Subramanian RB: *Withania somnifera* (Ashwagandha): a novel source of L-asparaginase. Journal of Integrative Plant Biology 2009; 51(2): 201-206.
14. Verma N, Bansal M and Kumar S: Whole cell based miniaturized fiber optic biosensor to monitor L-asparagine. Advances in Applied Science Research 2012; 3: 809-814.
15. Kumar K, Punia S, Kaur J and Pathak T: Development of plant asparagine biosensor for detection of leukemia. Journal of Pharmaceutical and Biomedical Sciences 2013; 35: 1796-1801.
16. Kataria M, Kaur N, Narula R, Kumar K, Kataria S and Verma N: L-Asparaginase from novel source- *Solanum nigrum* and development of asparagine biosensor. The Pharma Innovation Journal 2015; 4:81-84.
17. Oza VP, Parmar PP and Subramanian RB: Anticancer properties of highly purified L-asparaginase from *Withania somnifera* L. against acute lymphoblastic leukemia. Applied Biochemistry and Biotechnology 2010; 160(6): 1833-1840.
18. Sarin B, Verma N, Martín JP and Mohanty A: An overview of important ethnomedicinal herbs of *Phyllanthus species*: Present status and future prospects. The Scientific World Journal 2014; 2014: 1-12.
19. Zhao T, Sun Q, Marques M and Witcher M: Anticancer Properties of *Phyllanthus emblica* (Indian Gooseberry). Oxidative Medicine and Cellular Longevity 2015; 1-7.
20. Jain R, Pandey R, Mahant RN and Rathore DS: A review on medicinal importance of *E. officinalis*. International Journal of Pharmaceutical Sciences and Research 2015; 6(1): 72-84.
21. Patel JR, Tripathi P, Sharma V, Chauhan NS and Dixit VK: *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. Journal of Ethnopharmacology 2011; 138(2): 286-313.
22. Ahmad MS, Bano S and Anwar S: Cancer ameliorating potential of *Phyllanthus amarus*: *In-vivo* and *in-vitro* studies against Aflatoxin B1 toxicity. Egyptian Journal of Medical Human Genetics 2015; 16(4):343-353.
23. Giribabu N, Rao PV, Kumar KP,Muniandy S, Rekha SS and Salleh N: Aqueous extract of *Phyllanthus niruri* leaves displays *in-vitro* antioxidant activity and prevents the elevation of oxidative stress in the kidney of streptozotocin-induced diabetic male rats. Evidence-Based Complementary and Alternative Medicine 2014; 2014:1-10.
24. Mostofa R, Ahmed S, Begum MM, Rahman MS, Begum T, Ahmed SU, Tuhin RH, Das M, Hossain A, Sharma M and Begum R: Evaluation of anti-inflammatory and gastric anti-ulcer activity of *P. niruri* L. (Euphorbiaceae) leaves in experimental rats. BMC Complementary and Alternative Medicine 2017; 17: 267.
25. Huang ST, Pang JHS and Yang RC: Anti-cancer effects of *Phyllanthus urinaria* and relevant mechanisms. Chang Gung Medical Journal 2010; 33(5): 477-487.
26. Zhong MG, Xiang YF, Qiu XX, Liu Z, Kitazato K and Wang YF: Natural products as a source of anti-herpes simplex virus agents. Royal Society of Chemistry Advances 2013; v 3(2): 313-328.
27. Meister A, Levintoh L, Greenfield RE and Abendeschein PA: Hydrolysis and transfer reactions catalyzed by omega-amidase preparations. Journal of Biological Chemistry 1956; 215(1): 441-460.
28. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951; 193:265-275.
29. Scopes KR: Protein purification: Principles and practice. Narosa publishing house, Third Edition 1994.
30. Lineweaver H and Burk V: The determination of enzyme dissociation constants. Journal of the American Chemical Society 1934; 56(3): 658-666.
31. Singh A, Verma N and Kumar K: Screening and identification of medicinal plants for L-Asparaginase production. International Journal of Recent Scientific Research 2017; 8(11): 22029-22034.
32. Chagas EP and Sodek L: Purification and properties of asparaginase from the testa of immature seeds of pea (*Pisum sativum* L.). Brazilian Archives of Biology and Technology 2001; 44(3): 239-245.
33. Verma N, Kataria M, Kumar K and Saini J: Comparative study of L-asparaginase from different cytotypes of *W. somnifera* (L.) Dunal and its purification. Journal of Natural Products and Plant Resources 2012; 2(4): 475-481.
34. Mohamed SA, Elshal MF, Kumosani TA and Aldahlawi AM: Purification and characterization of asparaginase from *Phaseolus vulgaris* Seeds. Journal of Evidence Based Complementary and Alternative Medicine 2015; 2015:1-6.
35. Chang KS and Farnden KJF: Purification and properties of asparaginase from *Lupinus arboreus* and *Lupinus angustifolius*. Archives of Biochemistry and Biophysics 1981; 208(1):49-58.
36. Willis RC and Woolfolk CA: Asparagine utilization in *Escherichia coli*. Journal of Bacteriology 1974; 118(1): 231-241.

37. Kamble VP, Rao RS, Borkar PS, Khobragade CN and Dawane BS: Purification of L-asparaginase from a bacteria *E. carotovora* and effect of a dihydropyrimidine derivative on some of its kinetic parameters. Indian Journal of Biochemistry and Biophysics 2006; 43(6): 391-394.
38. Kumar S, Dasu V and Pakshirajan K: Purification and characterization of glutaminase-free L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. Bioresource Technology 2011; 102(2): 2077-2082.
39. Ali EMM: Purification and characterization of *Vigna unguiculata* cultivar asparaginase. Egyptian Journal of Biochemistry and Molecular Biology 2009; 27(1): 145-162.

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