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Bioprospecting of Proteases Enzyme of Some Indigenous Euphorbian Medicinal Plants

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Euphorbiaceae is among the large flowering plant families consisting of a wide variety of vegetative forms some of which are of great importance. Euphorbiaceae has traditionally delimited is one of the largest families of flowering plants, composed of over 300 genera and 8,000 species. This family is very diverse in range, composed of all sorts of plants ranging from large woody trees through climbing lianas to simple weeds that grows prostrate to the ground. Members of this family are widely distributed all around the world constituting both old world and new world plants some of which are yet to be identified. Many family members are inhabitants of tropical climates surviving hot dry desert conditions, while others are rainforest trees and herbs (Mwine and Damme, 2011).

Many cultures around the world have used members of the Euphorbiaceae family for its be lived medicinal properties. Because many of the world's people are founding less developed areas than typical, they have observed different "cures" for different ailments. Euphorbiaceae members have been used in treating gonorrhea, asthma, spleen enlargement, as well as tumors. A detail report on Ethnobotanical Euphorbian plants of North Maharashtra Region is given by Adsul et al., (2013) pertaining to use of them in treating various ailments. Euphorbiaceae is a complex family with a lot of research potential, Latex production is unique property of Euphorbian plants. Economic importance of these plants is also for possible biofuel production (Domsalla et al., 2010). Some plants are wild, xerophytic and succulent, other are agricultural crops and weeds, while some are ornamental (Arima et al., 2000; Patil, 2003; Badgujar,2011).

Plant Latex is a natural plant polymer secreted by highly specialized cells known as laticifers. Latex is a milky fluid secreted by duct of laticiferous tissue (Hagel et al., 2008), and mainly flow inside laticifers including root stem, leaves and fruits of all flowering plants. It is an emulsion like sticky material that exudes from various plant parts after having a small tissue injury. In most plant species latex is squirt out as white glue from bark of plants. It is a complex mixture of proteins, alkaloids, starch, sugars, oils, tannins, resins and gums. The latex is a milky fluid and a mixture of compound such as phytosterols, amine oxidases, protein, lipases and others (Mahajan and Badgujar, 2010). The latex has also been widely understood to act as a defense for the plants against generalist herbivores (Arribere et al., 1998; Charles and Kokati, 2011; Anusha et al., 2013).

Proteases refer to a group of enzymes whose catalytic function is to hydrolyze peptide bond of proteins. These enzymes are widely distributed nearly in all plants, animals and micro-organisms. They perform various biological functions in plants and particularly in humans they participate zymogen activation, fibrinolysis, complement activation, blood coagulation and digestion process. They also have commercial importance in food, leather and textile industry and therefore, they have become the focus

of wide range of industrial and medical application (Chetia et al., 1999; Dayanand, 2013). In mammalian physiology, proteases play an important role in the pathophysiology of several diseases such as asthma, cancer, AIDS and wound healing process. At the moment several examples of the use of enzymes in plants latex specifically proteases in different areas from the industry can be mentioned, modified proteins for the food industry, baking, beer elaboration, cheese production, detergent dust preparation, treatment of industrial effluents, textile industry, manufacture of leather, pharmaceutical industry, cleaning of surgical supplies, and biomedical (Abhijit Ray,2012).

A number of proteases from latex bearing species have been isolated and characterized (Lynn and Clevette, 1986; Rawlings et al., 2004; Yadav et al., 2011; and Khan and Toor, 2013). In addition, Badgujar and Mahajan, (2009) reported the proteolytic and milk clotting enzyme are present in different latex bearing plants of North Maharashtra region. Plant latex is an important source of proteases and therefore numbers of latex plants have been investigated for their protease activity and rennet action.

The increasing demand for cheese, the insufficient supply, high cost of rennet and associated ethical issues have led to the search for a suitable alternative to rennin. In recent years, growing research interest has been directed towards finding several new proteases from plants with milk coagulation potential. Plants are used as a source of proteases due to its easy availability efficient crude enzyme purification processes and isolation of natural coagulant (Arima and Iwaski, 1970; Chary and Reddy, 1983; and Kumari et al., 2010).

Scientific research that looks for a useful application, process, or product in nature is called biodiversity prospecting, or bioprospecting. In many cases, bioprospecting is a search for useful organic compounds in plants, microorganisms and fungi that grow in extreme environments, such as rainforests, deserts, and hot springs. For example, many scientists believe that the cures for AIDS, cancer and other diseases lie hidden beneath this green vegetation. Modern-day bio prospecting is simply an extension of our long history of exploring nature to improve the quality of our life. Biodiversity prospecting is nothing new. In fact, people have been bioprospecting since the dawn of civilization. Bioprospecting began when prehistoric people noticed that one plant root tasted better than another, or that some plants could be used as medicines. Later, scientists identified the active ingredients in these plants. People learned that the delicious plant root had higher sugar content, or they discovered that a plant used for medicine contained aspirin (salicylic acid). Bio prospecting involves searching for, collecting and deriving genetic material from samples of biodiversity that can be used in commercialized pharmaceutical, agricultural, industrial or chemical processing and products. In other words, it is the exploration of wild plants, microorganisms and animals for commercially valuable genetic and bio-chemical resources (Joshi et al., 2007).

Euphorbiaceae is among the large flowering plant families consisting of a wide variety of vegetative forms, and some of them are of great importance. It is need to explore traditional medicinal knowledge of plant materials belonging to various genera of Euphorbiaceae available in North Maharashtra State. Plants have always been the source of food, medicine and other necessities of life since the origin of human being. Plants containing ethno medicinal properties have been known and used in some forms or other tribal communities of Satpuda region (Adsul and Mahajan, 2013).

More than 110 latex bearing plants in different families are known to contain at least single proteolytic enzyme. In available literature has only 20 indigenous species of genus Euphorbia are known to contain proteolytic enzyme and out of them 10 are characterized as serine protease. The others are not characterized as stated in earlier part of thesis in context to this property (Nath and Dutta, 1998; Domsalla and Melzig, 2008, Naidu, 2011, Shah et al., 2014, Chavan et al., 2015).

In the present study, we determined proteolytic and milk clotting activity of 20 various laticiferous plants of Euphorbiaceae family. The earlier researchers have investigated the proteolytic and milk clotting activity of latex bearing plants of different families and their observations are good agreement to our results. The promising Euphorbian plants are Euphorbia tirucalli, Euphorbia neriifolia, Euphorbia prunifolia and non Euphorbian genus are Pedilanthus tithymalodies and Synadenium grantii. The proteolytic activity in the latex of them are 48±0.3 (Euphorbia tirucalli), 47.11±0.5 (Euphorbia neriifolia),

42.7±0.8 (Euphorbia prunifolia) 52.4±0.51 (Pedilanthus tithymalodies) and 24.8±0.3 (Synadenium grantii) U/g latex. This means that proteolytic activity of latex varies from 24.8 to 52.4 U/g. Latex of these plants usually contain serine protease except Pedilanthus tithymalodies. An occurrence of serine protease is common in the latex of Euphorbian plants. Earlier authors investigated proteolytic activity in the latex of Euphorbia tirucalli (46±0.8), Euphorbia neriifolia (45.11±0.5), Pedilanthus tithymalodies (47.12±0.3) and Synadenium grantii (28.21±0.12) U/g respectively (Yadav et al., 2011; Patel et al., 2012; Moro et al., 2013 and Dayanand, 2013).

Latex exhibit phytotoxicity against invertebrate and vertebrate animals mainly due to these secondary metabolites like saponin and alkaloid (Mwine and Damme, 2011). The observation of larvicidal activity of EPCF and the mortality rate of larvae is similar to evaluation of larvicidal effects of leaf and stem bark extracts of Jatropha curcas, Citrus grandis and Tinospora rumphii against the larvae of the dengue-vector, Aedges aegypti, (Deepa et al., 2015).

Mollucicidal activity of Euphorbia prunifolia shows poor results on physiological behavior and mortility rate on Biomphalaria glabarata. Our result is similar to earlier reported observation of Biomphalaria glabrata on crude latex of Euphorbia milii (Yadav and Jagannadham, 2008). Further Lima et al., (2012) confirmed the same experimental observations and physiological changes of Biomphalaria glabrata in latex of Euphorbia milii.

From results it is concluded that the crude enzyme of Euphorbia prunifolia latex showed noticeable effect in Anthelmintic activity on Pheritima posthuma as test worms. Similar observations are found in Carica papaya using same animal (Kanthal, 2012). The enzyme of Jatropha curcas has shown better Anthelmintic activity as compared to our results (Parmar et al., 2014). Nematicidal activity of EPCF is weak indicating that type of phyto ingredient(s) to kill larvae of this nematode is not potential.

The various authors used plant latex enzyme to produce cheese from milk with aim to find rennet substitute, (Badgujar and Mahajan, 2009; Yadav et al., 2006 and Pandey et al., 2012). The results revealed that, nutritional quality of cheese prepared from milk clotting enzymes of EPCF is not fully matched with Amul Cheese. Our results are similar with plant proteases employed for cheese production in various areas of the world include papain, bromelin, ficin, cucumisin, Sodom apple and Jacaratia corumbensis (Aworh and Nakai, 1986; Bhattacharya, 2008; Arima et al., 2008; Duarte et al., 2009 and Amri et al., 2012). The present results indicate the quality of cheese produce does not match with the commercial cheese. Why this happened? The answer is probably lack of strong aspartic enzyme in the latex (Yadav et al., 2011).

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