Plant Archives Vol. 22, No. 1, 2022 pp. 308-319 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2022.v22.no1.049

ASPERGILLUS NIGER BIOTIC ELICITORS OF SECONDARY PHARMACEUTICAL METABOLITES IN MEDICINAL PLANTS (*IN VITRO*): A REVIEW

Dhuha Sabah Nadir
Diyala General Directorate of Education, Baladruz, Iraq.
Email : dhuha.s.nader@gmail.com
(Date of Receiving : 12-08-2021; Date of Acceptance : 19-11-2021)

ABSTRACT

Aspergillus niger one of the most common and important fungal species It is; most commonly found in mesophilic environments such as decaying vegetation or soil and plants. Genome sequencing of *A. niger* as a biotic elicitorsis important because of its involvement in producing citric acid as well as industrial enzymes, such as amylases, proteases, pectinases and lipases. The use of these enzymes are essential because of its importance for transformation to food enzymes. Other properties of *A. niger* include of pharmaceutically significant secondary metabolites or phytopharmaceuticals such as alkaloids, glycosides, flavonoids, volatile oils, tannins, resins and aflatoxin. Metabolite production, involvement in food spoilage, and simply being a pathogen creates a great economic impact. Most of these secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is either extremely difficult or economically infeasible. Plants and/or plant cells *in vitro*, show physiological and morphological responses to microbial, physical or chemical factors which are known as 'elicitors'. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness. Here, we discuss the classification of *Aspergillus niger* as a biotic elicitors, for the production of secondary pharmaceutical metabolites from medicinal plants *in vitro*.

Keywords: *Aspergillus niger*, biotic elicitors, secondary pharmaceutical metabolite, medicinal plants, *in vitro*.

Introduction

Aspergillus niger

Aspergillus, one sizeable genus belonging to Aspergillaceae family, comprises as many as 492 species registered on the database of the National Center for Biotechnology Information (NCBI) to date. Its section Nigri is an important group of species, and the *A. niger* aggregate represents its most complicated taxonomic subgroup with eight morphologically indistinguishable taxa (Perrone *et al.*, 2011). Owing to superior survivability and adaptability, *A. niger* is ubiquitous in nature, including in terrestrial soil (Xie *et al.*, 2006), ocean (Li *et al.*, 2016; Uchoa *et al.*, 2017), the Arctic (Singh *et al.*, 2011), and space. It also occupies a wide spectrum of habitats in plants and animals such as herb(Shreelalitha and Sridhar, 2015; Manganyi *et al.*, 2018), shrub (Kaur *et al.*, 2015; Liu *et al.*, 2016), tree (Soltani and Moghaddam, 2014; Wang *et al.*, 2019), lichen (Elissawy *et al.*, 2019), shrimp (Liu *et al.*, 2013; Fang *et al.*, 2016), and marine sponge (Takano *et al.*, 2001; Hiort *et al.*, 2004). *A. niger* strain grows well in various media with different carbon sources, including glucose, bran, maltose, xylan, xylose, sorbitol, and lactose (Toghueo *et al.*, 2018). *A. niger* is not only a xerophilic fungi (mold that doesn't require free water for growth, can grow in humid environments), but is also a thermotolerant organism (capable of growing at high temperatures). Because of this property, the filamentous fungi exhibits a high tolerance to freezing temperatures. (Schuster *et al.*, 2002).The production of some secondary metabolites compounds can be induced by exposing the plant to a biotic or abiotic factors (Baldi and Bisaria, 2009). Fungi were one type of biotic elicitor and *Aspergillus niger* fungi was one of fungi that used in this field (Bashir *et al.*, 2006). *A niger*cell wall works as a polysaccharide elicitor, which induces calcium concentration in the cell and activates various defense responsive pathways leading to the accumulation of phytoalexins and low molecular weight antimicrobial compounds (Cordell 1997). *A. niger* produces most of the world's citric acid, a common preservative for foods, detergents, and industrial products. Many common foods and beverages, such as soy sauce, chocolate, soft drinks, vitamins, black tea, and fruit juice undergo a fermentation process with *Aspergillus* has been a very important microbe used in the field of biotechnology. Also, many of the enzymes produced by *A. niger*, such as, amylases, lipases, cellulases, xylanases and proteases, are considered GRAS (generally recognized as safe) by the United States Food and Drug Administration and is excused from the Federal Food, Drug, and Cosmetic Act food additive tolerance requirements. Even though it is considered GRAS, *A. niger* still must be treated safely and with care. The important use of "*Aspergillus*" secondary metabolites can be seen in several human medical applications such as the antibiotic penicillin (Gibbons and Rokas 2013). These human applications largely represent the use of recombinant genetics technologies and other bioengineering techniques.

Elicitors

Plants or plant cells *in vitro*, show physiological and morphological response to microbial, physical or chemical factors which are known as 'elicitors'. An 'elicitor' may be defined as a substance which, when introduced in small concentrations to a living cell system, initiates or improves the biosynthesis of specific compounds. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival persistence and competitiveness. The application of elicitors, which is currently the focus of research, has been considered as one of the most effective methods to improve the synthesis of secondary metabolitesor phytopharmaceuticals in medicinal plants, which include alkaloids, glycosides, phenols, flavonoids, volatile oils, etc. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavours and other industrial materials. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Commonly tested chemical elicitors are salicylic acid, methyl salicylate, bezoic acid, chitosan and so forth which affect production of phenolic compounds and activation of various defense-related enzymes in plants. Plants are challenged by a variety of biotic stresses like fungal, bacterial or viral infections. This lead to the great loss to a plant yield. Depending on this principle, some strategies were developed and used to encourage the *in vitro* production of secondary metabolites, these strategies include treatment with microbial, physical and chemical agents known as elicitors (Yue *et al*., 2016). It became known now that the use fungi is considered to be one of the best biotic elicitors. Because it stimulate the plant's cells to produce the secondary metabolites such as flavonoids and phenols as in the case of using the fungus *A. niger* as a biotic elicitor (Shanker and Shanker, 2016; Ibrahim *et al.,* 2019). It also plays an important role in the field of biotechnology to produce chemical substances, enzymes and medical drugs (Baker and Bennett, 2008).Classification of biotic elicitor for the production of secondary metabolites directly released by microorganisms and recognized by the plant cell (enzymes, cell wall fragments),formed by action of microorganisms on plant cell wall (fragments of pectins etc.), formed by the action of plant enzymes on microbial cell walls (chitosan, glucans)and compounds, endogenous and constitutive in nature, formed or released by the plant cell in response to various stimuli (Namdeo, 2007).

Medicinal Plants

Medicinal plants are the source of bioactive compounds with many blockbuster drugs derived directly or indirectly from plants having therapeutic value. The production of secondary metabolites in plants is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Rao *et al*., 2002; Thakur *et al*., 2013).To overcome this problem we can preserve the resulting loss of biodiversity by minimizing use of the product from the intact plant and synthesize secondary metabolite production using *in vitro* techniques by elicitors for enhancing their bio-production to meet commercial demands.

Medicinal plants of pharmaceutical interest whose secondary metabolite production is enhanced by the addition of the extract of biotic elicitor

Aspergillus niger

Ruta graveolens L. is perennial medical plant belongs to the family Rutaceae (Al- Kateb, 2000), widely distributed and has a long history in traditional medicine, as it had been used in medicine for more than 1500 year ago (Bowen *et al.,* 1988). Poutaroud *et al.* (2000) mentioned that the plant has a strong smell and produce different types of secondary metabolites, that give it the medical and pharmaceutical importance, like essential oils, alkaloids, flavonoids and furanocoumarins (Al- Mahdawe, 2018a). It was also reported that it contains carotenoids, chlorophylls and a number of compounds that have antimicrobial activities such as acridone (Wessner *et al.,* 1999).Talfan *et al*., (2020), indicated that the addition of *A. niger* extract, has a strong effect on increasing the concentration of Psoralen, Xanthotoxin and Bergapten in the callus cultures. The effect of the addition of *A. niger* extract on increasing the production and accumulation of furanocoumarins in callus culture. Could be explained according to the fact that plant cells give their highest production when they are under stressful conditions or at study state or clumped together. There is a number of important factors that affect callus production and accumulation of secondary metabolites, such as, the elicitor concentration, the length of exposure to the elicitor, the formation of nutrients, the age and the culture status. Ibrahim *et al.* (2019), mentioned that the addition of *A.niger* extract at a concentration of 2.0 ml L^{-1} to callus culture of the plant *Calendula officinalis* L., produced the highest concentration of salicylic acid 1.147 mg g^{-1} in comparison of the control treatment which reached 0.428 mg g^{-1}. Manjula and Mythili (2012), also reported that the addition of *A. niger* extract at a concentration of 2.0 ml L-1 to callus cultures of the plant *Marsilea quadrifolia*, resulted in an increment in the growth of the plant and the concentration of carbohydrate and protein as primary metabolites, which reflected later on increment of the accumulation of the secondary metabolites, such as, the phenolic substances and flavonoid in the roots and the branches.

Psoralea corylifolia L. is an important medicinal plant found in the tropical and subtropical regions of the world. It synthesizes diverse phenylpropanoids such as furanocoumarins, isoflavonoids etc. (Boardley *et al*., 1986). Psoralen is the furan ocoumarin and commercially important for having broad range of pharmacological activities such as photosensitizing, photobiological and phototherapeutic properties (Frank *et al*., 1998). Psoralen has been used for the photochemotherapy of vitiligo and skin diseases such as psoriasis, mycosis fungoides and eczema (Khushboo *et al*., 2010; Ozkan *et al*., 2012). It also shows antitumor (Szliszka *et al*., 2011), antibacterial (Chanda *et al*., 2011) and antifungal properties (Srinivasan and Sarada, 2012).The addition of *A. niger* elicitor to the cultured cells of *P. corylifolia* L. increased the psoralen accumulation. The maximum increase in psoralen accumulation was recorded in 20 days old culture and it decreased with increase in age of the culture thereafter. The accumulation of psoralen increased with increase in age of cell culture up to 16 days and thereafter the increase was inconsistent (Syed and Mirza 2014). The stimulation of psoralen accumulation by biotic elicitors such as *A. niger*, *Penicilliumnotatum*, yeast extract and chitosan has also been observed in the cell cultures of plant species viz. *Calendula officinalis* (Wiktorowska *et al*., 2010), *Sorbus aucuparia* (Gaid *et al*., 2011) and *Abrus precatorius* (Karwasara *et al*., 2010, 2011). The cell wall extract preparation of *A. niger* possessed an oligosaccharide elicitor that induced high level of shikonin (Wen and Ri-qiang, 1996). Another significant effect of the elicitors observed in the experiments was the rapid increase in psoralen accumulation with elicitor dosage. Thus, the accumulation of psoralen is a dose elicitor dependent response of *P. corylifolia* L. cell cultures. The growth and accumulation of secondary metabolites were influenced by the type and mode of elicitor preparation (Karwasara *et al*., 2011). Syed and Mirza, (2014), mentioned that the extract of *A. niger* (1.0% v/v)was found to be the best for maximum metabolite elicitation influenced the accumulation of psoralen in the cultured cells.

Calendula officinalis L. is one of the plant of Asteraceae family, it is an aromatic plant that is classified in terms of its growth into annual winter ornamental plants (Alexopoulos, 1962). The plant has extensive uses in the field of herbal medicine flowers are used in the treatment of smallpox, measles, jaundice, constipation and reduced bleeding during menstruation (Al-Taha and Al-Mazine, 2016). Ibrahim (2019), indicated that addition of different concentrations of *A. niger* fungus extract, it has an effect on increasing the accumulation of salicylic acid in the callus, *of Calendula officinalis* which increased by increasing the concentration of fungus extract, and reached the highest content at the concentration2.0 mg. L^{-1}.

Andrographis paniculata Nees. is commonly known as 'Kalmegh' in India and as a medicinal plant belongs to the family Acanthaceae. The plant is recommended for its drug utility in Indian Pharmacopoeia and widely used in Ayurveda, Unani, Siddha and Homeopathy systems of medicines. The plant is reported to possess terpenoids and flavonoids. The major terpenoids viz. 14-deoxy-11-oxoandrographolide, 14-deoxy-11, 12-didehydroandro-grapholide and 14-deoxyandrographolide andrographolide are the active constituents of this plant. The main active constituent is andrographolide which is reported to possess liver stimulant, astringent, anodyne, tonic and alexipharmic properties and useful in dysentery, cholera, diabetes, consumption, influenza, bronchitis, swellings, itches, piles and gonorrhea (Zhao and Frang 1991). The most significant pharmaceutical properties of this plant are anticancerous (Kumar *et al*. 2004) and anti-HIV (Calabrese *et al*. 2000). Moinuddin and Vijay (2013), showed that elicitation of andrographolide by *Aspergillus niger* elicitors in cell suspension culture of *Andrographis paniculata*, in 4 days and 7 days treatment duration, 1 ml of *A. niger* extract was found to be most positive concentration for eliciting andrographolide compound. The estimated quantity of andrographolide was 52.0 µg/g and 331.0 µg/g in 4 days and 7 days treatment duration, respectively, which showed 2.47 and 3.76 fold increase over their respective controls.

Datur metel L. (Solanceae), a small branched perennial herb with purple coloured flowers, is distributed in the tropical and sub-tropical regions of the world. This medicinal plant has been traditionally used as intoxicant, emetic, digestive and healing since ancient times (Anonymous, 1952; Muthukumar *et al*., 2004). The main active constituents of the plant are medicinally important tropane alkaloids-hyoscyamine and scopolamine.*D. Metel* showed very high content of hyoscyamine (Knopp *et al*., 1988) and scopolamine (Hiraoka *et al*., 1996). Being antichlolinergic agents, these are used in medicine as antispasmodics, preoperative medication, analgesics, narcotics, sedatives and in treatment of asthma, Parkinson's disease and motion sickness (Pitta-Alvarez *et al*., 2000). The use of elicitors is one of the effective strategies employed to increase the production of important alkaloids in cell and organ culture. L Ajungla *et al*. (2009) mentioned that root cultures treated with 1.0 g L^{-1} of *A. niger* homogenate resulted in higher hyoscyamine (1.77 mg/g dw) and scopolamine (0.087 mg/g dw) production than that the *Alternaria* sp. and *Fusarium monoliforme* and the results indicate that the *A. niger* homogenate is favourable for promoting of tropane alkaloids in *D. metel*.

Hypericum perforatum L. (Hypericaceae) known as St John's wort is an important medicinal plant. A number of pharmacological studies and clinical trials have shown that *H. perforatum* extracts possess an astounding array of pharmacological properties including antidepressant, anti-inflammatory, antiviral, anticancer and antibacterial activities. These medicinal properties are related to the composition of the secondary metabolites present in the extract, particularly hypericins, hyperforins, flavonoids, xanthones and other valuable compounds (Wang *et al*., 2012). Xu *et al*. (2005), indicated that *A. niger* cell walls induced hypericin biosynthesis productionin *H. perforatum* cell suspension cultures and found that, fungalelicitor prepared from the *A. niger* induces multiple responses of *Hypericum perforatum* cells, including nitricoxide (NO) generation, jasmonic acid (JA) biosynthesis. Also, Gadzovska *et al*. (2015) reported a significant increase in naphtodianthrones, total phenolics, flavonoids, and anthocyanins in *Hypericum perforatum* cell suspensions by adding *A. niger* extract.

Gymnema sylvestre, native to central and western India, tropical Africa, and Australia, is a perennial woody climber rich in triterpenoid saponins belonging to the oleanane (gymnemic acids) and dammarene (gymnemasides) classes (Parijat *et al*., 2007). The gymnemic acids are a group of closely related molecules isolated from the leaves of *G. sylvestre* (Liu *et al*. 1992; Manni and Sinsheimer 1965). The anti-diabetic, anti-sweet and anti-inflammatory activities of *G. sylvestre* have been attributed to the presence of gymnemic acids; the other phytoconstituents include flavones, anthraquinones, hentriacontane, resins, d-quercitol, lupeol, b-amyrin-related glycosides, and stigmasterol. The cell wall acts as a chemical messenger with specific regulatory properties. The results obtained by Bhuvaneswari (2013), showed that the maximum accumulation of gymnemic acid was observed with *A. niger*, i.e. 11.2-fold (98.65 ± 0.93 mg/gDCW). This is 1.6-fold higher than the abiotic elicitor $CdCl_2$.On the other hand, Subathra and Mohana (2011), mentioned that when *A. niger* cell extract was used as a elicitor, the productivity of gymnemic acidincrease was considerably greater than that obtained with non-elicited cultures.

Glycyrrhiza. glabra L. plant belongs to Fabaceae family and is the inhabitant of Central and Southwest Asia. *G. glabra* is commonly known as Jothi-madh, Mulhatti (Hindi), licorice, liquorice, sweet wood (English) (Jatav *et al*., 2011). *G. glabra* plant is blessed with many medicinal properties. The use of licorice is more than 4000 years old. It is considered under important medicinal plants mentioned in

Assyrian herbal (2000 BC). This plant is used in the treatment of dyspepsia, gastric ulcers, fevers, liver ailments, asthma, bronchitis, sore throats, Addison's disease, and rheumatoid arthritis. It is also useful as an antitussive, expectorant, and laxative. In ancient times, this plant was also suggested in cases of women sterility. Licorice root is considered under top five herbs, which are recommended for the treatment of fatigue. This herb decreases temptation for sugars and increases cortisol activity in the human body. Glycyrrhizin is present in a very high amount in licorice roots. The roots of licorice contain a large amount of glycyrrhizin (up to 15%) and oleanane-type triterpene saponins. These saponins are used in various foods and industrial, cosmetic, and pharmaceutical applications. Saponins are commercially used in food industry as foaming, detergent, emulsifying, wetting, and sweetening agents (Hostettmann and Marston, 2005; Shibata, 2000). The pharmacological properties of triterpenes have been broadly studied which showed that these compounds have significant medicinal properties. Besides this, they also showed involvement in plant defense responses. Glycyrrhizin is also efficient against several viruses, such as HIV (Ito *et al*., 1987, 1988) and severe acute respiratory syndrome (SARS caused by corona virus-like viruses) (Cinatl *et al*., 2003). It is used for curing acute respiratory problems, gastritis, gastric ulcers, inflammatory conditions in general, and adrenal exhaustion. Compounds found in licorice roots possess both estrogenic and antiestrogenic activity, and due to these properties, this important herb is used for treating the female hormonal problems (Jatav *et al*., 2011). Although some side effects are also associated, due to high doses and prolonged use of this, such as hypokalemia, hypertension, mineralocorticoid effects, myoglobinuria, lethargy, quadriplegia, etc. (Nasrollahi *et al*., 2014). To improve the yield of glycyrrhizin, some fungal elicitors prepared from *Aspergillus niger* and *Rhizopus stolonifer* were tested at different concentrations in transformed cell suspension cultures of *A. precatorius*. The maximum enhancement of 4.9- and 3.8-fold in glycyrrhizin contents was obtained with *A. niger* (7.5% v/v) and *R. stolonifer* (5.0% v/v), respectively, on the fifth day after elicitor treatment (Karwasara *et al*., 2011).

Bacopa monnieri is a medicinal plant belongs to *Plantaginaceae* family commonly known as Brahmi, found throughout the Indian subcontinent in wet, damp and marshy areas. It is used in traditional Indian medicine and Ayurveda for the treatment of anxiety and improving intellect memory in several countries. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative free radical scavenging and anti-lipid peroxidative activities. The use of whole plant system for medicine, poor replenishment efforts and untrained plucking of the plant material leads this medicinal plant towards endangered. The major chemical entity shown to be responsible for neuropharmacological effects and the nootropic action or antiamnesic effect of *Bacopa monnieri* is Bacopasaponins A, B, and C which are dammarane-type triterpenoid saponins. Since the supply is limited and faces constraints in meeting the increasing demand of these biochemical. Pharmacological properties of *Bacopa monnieri* were studied extensively and the activities were attributed mainly due to the presence of characteristic saponins called as Bacosides. Bacoside have been indicated for memory-enhancing properties while Bacoside A assists in release of nitric oxide that allows the relaxation of the aorta and veins, to allow the blood to flow more freely through the body making this exceptional plant a nootropic drug. According to Central Drug Research Institute (CDRI) situated in Lucknow, the saponins, Bacosides A and B are responsible for repairing damaged neurons; furthermore *Bacopa monnieri* has been studied clinically for its acute and chronic effects on cognitive function. Plants have been found to elicit the same response as the pathogen itself when challenged by compounds of pathogenic origin (elicitors). Biotic elicitors have biological origin derived from the pathogen or from the plant itself. Misal *et al*. (2020), resulted that the *Aspergillus niger* filtrate significantly influenced the Bacoside production in Brahmi. After seven days elicitation, maximum enhancement in Bacoside (1.62% DW) was reported at lower concentrations of *Aspergillus niger* filtrate (0.5 ml/L) over control. Higher concentrations of *A. niger* filtrate also showed increasing but undulating results in Bacoside production over the control. Elicitation effects were might be due to the fungal cell wall works as a polysaccharide elicitor, which induces calcium concentration in the cell and activates various defense responsive pathways leading to the accumulation of phytoalexins and low molecular weight antimicrobial compounds (Cordell, 1997).

Panax ginseng is a perennial herb of the Araliaceae family, is well known traditional medicine plant and its root has been used as a herbal remedy for various disorders (Akerele, 1992). The herb is of pharmacological importance because of the presence of major bioactive compound triterpene saponin called Ginsenoside (Rahimi *et al*., 2015). Ginsenoside Rg3is not naturally produced in ginseng. Of particular note are the anti-tumor effects produced by ginsenosides. Ingredients of ginsenosides, such as Rb1, Rg1, Rg3, Rh2, PPT, and compound-K, have shown pharmacological effects through a variety of mechanisms (Li *et al*., 2008; Liu *et al*., 2000).In regards to Rh2 production, which has been shown toproduce anticancer effects, β-glycosidase purified from *Aspergillus niger* was shown to effectively produce ginsenoside F2 at a good yield (F2: 305 mg/g) (*Youl et al*., 2012). This process fully converted the compounds to F2 and prevented there action from proceeding further. Ginsenosides F1 and F2produced by β-glycosidase with high yield and the production of Rh2 from F2 are more favorable because the pathway from F2 to Rh2 was predominant as opposed to the pathway creating compound-K. (Yan *et al*., 2008; Yu*et al*., 2007).

Helicteres isora L. (Indian screw tree, a plant with traditional medicinal usages) has been reported as a cleaner source of diosgenin, where the compound is not admixed with other steroidal sapogenins (Barik *et al*., 1998; Deshpande and Bhalsing, 2014; Kumar *et al*., 2014). Exploration of this plant for diosgenin production is therefore advantageous. One more advantage of choosing this plant is that it is abundantly found in almost all parts of the country in forests as undergrowth, especially as secondary growth. This makes it a natural choice for exploration as a source of diosgenin. However, the diosgenin content is low in *H. isora* as compared to other traditional plant sources of diosgenin (Barik *et al*., 1998), which needs to be enhanced before commercial exploration of this plant as an alternative source of diosgenin. As stated earlier, plant cell cultures have been established as potent alternative sources for the production of high value secondary metabolites of industrial importance in a holistic (without causing destruction to the natural sources) and sustainable way (Rao and Ravishankar, 2002; Mulabagal and Tsay 2004; Hussain *et al*., 2012). Diosgenin, one of the most important plant secondary metabolites, is a steroidal sapogenin traditionally derived from the tubers of Dioscorea species (yams). It is a precursor of sex hormones (progesterone), corticosteroids (corticosone) and contraceptives as well as other important steroids (Zhang *et al*., 2009; Zhu et al., 2010; Wang *et al*., 2011; Selim and Al Jaouni, 2015; Sethi *et al*., 2018). It has also showed pharmacological activities such as anti-lipoperoxidative and anti-aging effects, cognitive impairment, hypoglycaemic effect, antifungal and antiviral activities (Jayachandran *et al*., 2009; Chiu *et al*., 2011; Wang *et al*., 2011; Patel *et al*., 2012; Hao *et al*., 2015; Sethi *et al*., 2018). In India, steroidal drug production is almost 100% based on diosgenin and diosgenin accounts for two-third of the total world consumption of steroids (Chaturvedi *et al*., 2007). Its annual global demand is 3000 tonnes; while in India, 150 tonnes of diosgenin are required per year, however, total production of diosgenin in India is only 30tonnes annually and rest is met by imports (Dangi *et al*.,2014; Deshpande and Bhalsing, 2015).The traditional sources of diosgenin are under threat due to their over-exploitation for extracting diosgenin, consequently, some of the species with high diosgenin contentsuch as *Dioscorea zingiberensis* and *D. deltoidea* are fast depleting (Chaturvedi *et al*., 2007; Li *et al*., 2012). This necessitates new alternative diosgenin sources and to develop strategies for its maximum, cost-effective production. In an attempt to identify an alternative and potent source of diosgenin. Samrin *et al*. (2020), study and focused on exploration of suspension cultures of *H. isora* for optimal production of diosgenin via biotic elicitation. Fungal elicitors from cultures of *Aspergillus niger* (ATCC10578) and *Saccharomyces cerevisiae* (NCIM3050) were prepared using fresh biomass. The results indicated that elicitor prepared using *A. niger* was responsible for significant increment in biomass production at all the applied concentrations (1%, 1.5% and 2%). Highest DW was observed at 1.5% fungal elicitor-treatment with a twofold increase over control. The same treatment was found responsible for highest diosgenin production (1.42-fold higher over controls).Fungal elicitors are considered as surface structures and/or fungal cell-secretions, with fungal mycelia or degraded fungal mycelial-products, and fermentation broth which may also contain fungal secretions. The said fungal elicitorhence may contain sugars (polysaccharides, oligosaccharides), proteins (glycolipid proteins, glycoprotein, and peptides), fatty acids and other substances. Fungal elicitorsoften result in biomass and secondary metabolite enhancement, as well as improved enzymatic activities in plants (Chen *et al*., 2015).

Ephedra alata L. is an Egyptian natural plant species found mainly in Sinai desert and Eastern Mediterranean coastal region (Boulos, 2009). It is a pharmaceutically important plant, which belongs to the Ephedraceae family of gymnosperms and is known to have a number of medicinal properties. *Ephedra alata* shows antimicrobial, antioxidant, and hypoglycemic activities (Soltan and Zaki, 2009; Parsaeimehr *et al*., 2010; Chebouat *et al*., 2014; Al-Snafi, 2017). In general, plants in the genus *Ephedra* havebeen used in traditional medicine to treat allergy, bronchialasthma, chills, cold, cough, edema, fever, flu, nasalcongestion, and headache (Parsaeimehr *et al*., 2010).Phytochemical analysis of *E. alata* indicated the presence of tannins, cardiac glycosides, alkaloids, phenolics, reducing sugars, and flavonoids (Jaradat *et al*., 2015). Additionally, *Ephedra* species contain alkaloids such asephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, methylpseudoephedrine, ephedroxane, and ephedradine A-D. Phenolic compounds including chlorogenic acid, rutin, catechin, quercetin, and coumaric acid and various flavonoids have also been isolated from *E. alata*. The total amount of alkaloids isolated from *E. alata* aerial parts was 0.2–0.22% (Al-khateeb *et al*., 2014), and the amount of ephedrine and pseudoephedrine was 0.05–0.19% and >0.5%, respectively (Al-Snafi, 2017). Ephedrine is a naturally occurring alkaloid in different species of *Ephedra*. It is used as a drug and has structure and activity similar to those of adrenaline, which raises blood pressure, heart rate, and respiratory capacity (Limberger *et al*., 2013). Ephedrine produces several pharmacological effects such as cardiovascular effect (it increases the arterial pressure by peripheral vasoconstriction and cardiac stimulation), bronchodilatation, nasal decongestion, mydriasis, nocturnal enuresis, spinal anesthesia, appetite suppressant and weight loss, cytotoxic effects, and many other (Al-Snafi, 2017). Most of the ephedrine produced today for medicaluse is obtained by chemical synthesis, because the process of extracting and isolating from the plant is difficult and economically costly (Limberger *et al*., 2013). Therefore, it is necessary to find an effective, practical, and economically feasible method to provide a continuous, natural, and pure source of ephedrine in large quantities as an alternative to the synthesized one that causes negative side effects. Therefore, attention should be given to isolate natural compounds instead of producing synthetic ones (Khan *et al*., 2017). In the present study, the *A. niger* extract was found to be useful for the enhancement of ephedrine accumulation. It was observed that treatment with the lowest concentration of *A. niger* extract caused the highest elicitation; thus, elicitor concentration and the exposure time are very critical for the elicitation of ephedrine production. This result agrees with that reported by Taha *et al*. (2009), who found that 0.25% of *A. niger* extract resulted in the production and accumulation of the highest amount of total alkaloids, vinblastine, and vincristine in the callus culture of *Catharanthus roseus*. However, as reported in the present study, increasing the concentration of *A. niger* extract showed less ephedrine accumulation. This may be because the mycotoxins have hindered ephedrine production and its biosynthetic pathway was negatively affected. Similar results were reported by Mathur (2018), who founda considerable decrease in secondary metabolite productionand growth of *Commiphora wightii*, *Zingiber officinale*, and *Daucus carota* cell suspension cultures when *A. niger* extract was used as an elicitor. In general, the accumulation of ephedrine was promising and varied largely according to the applied elicitors. Ephedrine accumulated in huge amounts as compared to that in the mother plant (7-fold) and the control treatment without elicitation after 24 days of exposure (6.427-fold). These results are very promising for the large-scale production of this valuable bioactive compound. The present investigation could be scaled up for the production of commercially feasible levels of ephedrine by using suspension cultures of *E. alata*.

Atropa belladonna L. (Solanaceae) is one of the most important medicinal plants and is a source of tropane alkaloids such as hyoscyamine and scopolamine. Medicinally, *A. belladonna* is used for the use of its alkaloids in the treatment of Parkinsons disease for its anti-inflammatory properties, for relief of bronchial asthma and motion sickness and its ability to counteract toxic agents. Belladonna extract is used as an antimuscarinic agent, which accounts for its use as a spasmolytic drug. Also, it is used as a concomitant therapy in the treatment of peptic ulcer and functional digestive disorders, including spastic, mucous, and pancreatitis. Taha, (2003) showed that The effect of different concentrations (0%, 5 %, 10%, 15 % and 20%) of 0.1P.C.V. of *A. niger* (0, 2.5, 5.0, 7.5 and 10 mg/mL of liquid cell cultures), which were added to the MS medium containing 1 mg/L of each of NAA and BA on cell number (105) and total alkaloid production from different types of cell cultures were investigated. Data shows that leaf explants of *A. belladonna* gave the optimum value for cell number (5.92 x105) with the blank elicitor treatment, as compared with elicitor treatments. The increase in elicitor levels reduced cell growth, but stimulated the accumulation of total alkaloids (calculated as scopolamine %). Stem cell cultures showed a low cell number (1.25 x 105) as well as low total alkaloid production. *A. niger* at 10 %after 10 days from the duration of incubation (21 days) showed the highest value fortropane alkaloid accumulation, in comparison to the other concentrations The highest values for total tropane alkaloids were 0.048 %, 0.035 % and 0.018% for leaf, root and stem cell cultures, respectively. The obtained results are in agreement with those of Harkes *et al*. (1985), who indicated that anthraquinone content can be increased to 500 µg/g fresh weight (ascompared with the control) by the addition of 0.5 mg/mL of *A. niger* as a biotic elicitor to the culture medium of *Cinchona lederiana* cell cultures. Most plant secondary products are produced in the stationary phase of cell growth. It may be concluded that cultivation of leaf explants of *A. belladonna* in liquidMS-medium containing 1mg/L of each of NAA + BA in the presence of 5 mg/mL of *A.niger* for 10 days was the most favourable condition for stimulating total tropane alkaloids production.

Oldenlandia umbellata L. is one of the important members of *Rubiaceae* known for its dyeing and medicinal properties. This plant is used in traditional medicine and Siddha for its styptic property (Seydel and Dornenbug, 2006). The leaf and root extracts were considered as good expectorants and used for treatment of asthma, bronchitis, and bronchial catarrh (*Gupta et al*., 2007). The decoction prepared from its leaves is used as a rinse to treat poisonous bites (Rekha *et al*., 2006), and also used as a febrifuge. A novel pH indicator dye was reported from this plant (Siva *et al*., 2009). Extract of the whole plant shows significant antitumor activity (Sethuramani *et al*., 2014). The major dyeing property depends on anthraquinone contents of roots and used to impart red color to the textile materials (Siva, 2007; Siva *et al*. 2012). The multipurpose usage has made increased usage of this plant and a reliable protocol was developed for enhanced growth of the plant through tissue culture technique. Saranya and Velayutham, (2019) mentioned that the treated calli were further subcultured on solid media with respective fungal elicitors and obtained large amount of calli. Of the three fungal elicitors treated, large amount of green compact callus was obtained on medium treated with the extract of *A. niger* followed by *T. viride*. However, the callus treated with *M. prayagensis* showed an equal response for callus growth to that of control at all tested concentration. The maximum shoot regeneration response was obtained from 100 µg l^{-1} *A. niger* elicitor treated calli. Maximum number of 78.8 shoots with shoot length of 11.4 cm was achieved on the callus treated with *A. niger*. The maximum root regeneration response was obtained from 100 µg l^{-1} *A. niger* elicitor treated shoots followed by 50 µg l^{-1} *M. prayagensis* and 25 µg l^{-1} *T. viride* with an average number of 46.6, 28.6 and 30.8 respectively from root induction medium containing 6 µM IBA. The root regeneration frequency and number of roots were decreased whenthe shoots were treated with above optimal concentration.

Blumea lacera (Burm.f.) DC. is a medicinal plant with strong odour of terpentine and it belongs to Asterceae family. In Ayurveda, *Blumea lacera* is described as anthelmintic, liver tonic, expectorant, thermogenic, anti-inflammatory, ophthalmic, digestive, antipyretic and memory enhancer (Warrier *et al*., 1996). The plant is astringent, diuretic and useful in catarrhal affections (Quisumbing, 1998). Essential oil has analgesic, hypothermic, tranquillizing and antimicrobial activity (Dixit and Verma, 1976; Bharnagar *et al*., 1977). Campestrol, triterpenoid and prenylated phenol glycosides are the main active constituents of *B. lacera* (Pal *et al*., 1972; Agarwal *et al*., 1995). The other important constituents are flavonoids (Rao *et al*., 1997), monoterpene glycoside (Ragasa *et al*., 2007). The essential oil of the plant include β-caryophyllene, thymol hydroquinone dimethyl ether, caryophyllene oxide, α-humulene and E-β-farnesene (Laakso, 1989) and coniferal alcohol derivative (Bohlmann and Zdero, 1969).Vijay *et al*., (2016), resulted that *Aspergillus niger* treatment with 1.5 ml concentration for 4 days duration revealed 3.3 fold enhancement in flavonoid content (0.036 mg/g) as compared to control (0.011 mg/g). The results indicate that for flavonoid elicitation in *Blumea lacera*, *Aspergillus niger* is more responsive than Salicylic acid.

Conclusion

The present review reports the information about the use of *Aspergillus niger*as biotic elicitorsin medicinal plants for the enhancement of their bioactive compounds by different *in vitro* culture techniques to meet the commercial demands of pharmaceuticals. It is found that medicinal plants are used across the globe to cure various diseases like, Parkinson's disease, motion sickness, hypertension, tumor, depression, constipation, malaria, asthma, jaundice, vitiligo, skin diseases such as psoriasis, mycosis fungoides, eczema, rheumatism, cancer and diabetes, etc. These plants have medicinal properties due to presence of a bioactive compound in them. The bioactive compound in the intact plant is less in quantity so to synthesize secondary metabolites in desired quantity. *Aspergillus niger* was used as biotic elicitors*in vitro* using different cultures.

Table 1: Effect of *Aspergillus niger* as biotic elicitors on secondary metabolites production of medicinal plants *invitro* culture.

Plant species	Elicitors	Secondary Metabolites	Type of Culture	References
Ruta graveolens	*A. niger*	furanocoumarins	callus culture	Talfan *et al.*
Psoralea corylifolia	*A. niger*	psoralen	cultured cells	Syed *et al.*
Calendula officinalis	*A. niger*	salicylic acid	callus	Ibrahim *et al.*
Andrographis paniculata	*A. niger*	andrographolide	in cell suspension culture	Moinuddin*et al.*
Datur metel	*A. niger*	alkaloids- hyoscyamine and scopolamine	cell and organ culture	L Ajungla *etal.*
Hypericum perforatum	*A. niger*	hypericin	cell suspension cultures	Xu *et al.*
Gymnema sylvestre	*A. niger*	gymnemic acid	Cell suspension cultures	Bhuvaneswari *et al.*
Glycyrrhiza. glabra	*A. niger& Rhizopus stolonifer*	glycyrrhizin	transformed cell suspension cultures	Karwasara *et al*
Bacopa monnieri	*A. niger*	Bacoside	full culture	Misal *et al.*
Panax gingseng	*A. niger*	ginsenoside	purified from A. niger	*Youl et al.*
Helicteres isora	*A. niger*	diosgenin	cultures	*Samrin et al.*
Ephedra alata	*A. niger*	Ephedrine	suspension cultures	Ghada *et al.*
Atropa belladonna	*A. niger*	alkaloid	cell culture	Taha
Oldenlandia umbellata	*A. niger*	anthraquinone	callus	Saranya *et al.*
Blumea lacera	*A. niger*	flavonoid	cell culture	Vijay *et al.*

References

Agarwal, R.; Singh, R.; Siddiqui, I.R.; Singh, J. (1995) Triterpenoid and prenylated phenol glycosides from *Blumea lacera*. *Phytochemistry*. 38: 935-938.

Akerele O. (1992). WHO guideline for assessment of herbal medicines. *Fitoterapia* 63: 99-104.

Alexopoulos, C.J. (1962). Introductory Mycology. 2th ed i. John wiley and sons, Inc. New York, London. 11:123-134.

Al-Kateb, Y.M. (2000). Plant taxonomy. 2nd edition Ministry of Higher Education and Scientific Research, University of Baghdad. Published by Dar-Alkutob, University of Mosul, Mosul, Iraq.

Al-khateeb E.; Al-Ani H.; Al-Kadi K.; Al-Obaidi E.D.F.; ShalanN.; Al-Rawi N. (2014) Investigation of the alkaloids of twoEphedra Spp. wildly grown in Iraq. *Jordan J. Pharm. Sci*., 7(3): 191–198.

Al-Mahdawe, M.M.; Al-Mallah, M.K. and Ahmad, T.A. (2018 a). Isolation and identification of rutin from tissues cultures Influence of the extract of biotic elicitor *Aspergillus niger* on the production of furanocoumarins in callus cultures of *Ruta graveolens* L. *J. Pharm. Sci. and Res.;* 10(6):1517-1520.

Al-Snafi A.E. (2017). Therapeutic importance of *Ephedra alata* and *Ephedra foliate* a review. *Indo Amer. J. Pharm. Sci*., 4(2): 399–406.

Al-Taha, H.A. and Al-Mazine, L.H.M. (2016). Effect of 2,4-D and BA on Induced callus of somatic embryos and adventitious shoots regeneration from leaves segments culture of dwarf *Gardenia jasminoides* Ellis. cv. redicans. *Basrah Journal of Agricultural Sciences*, 29(1): 214-230.

Anonymous, (1952). The wealth of India-A dictionary of Indian raw materials and industrial products (Publications & Information Directorate, CSIR, New Delhi) 3, 14-19

Baldi, A.; Srivastava, A.K. and Bisaria, V.S. (2009). Fungal elicitors for enhanced production of secondary metabolites in plant cell suspension cultures. *Symbiotic Fungi. Springer-Verlag, Berlin Heidelberg*, PP: 373–380.

Baker, S.E. and Bennett, J.W. (2008). The *Aspergilli*: Genomics, Medical Aspects, *Biotechnology and Research Methods, London*, 1: 3-13.

Barik, B.; Dey, A.K. and Das, P.C. (1998). *Helicteres isora* Linn, a new source of diosgenin. *Indian J Chem.,* 20(B):938

Bashir, S.; Janbaz, K.H.; Jabeen, Q. and Gilani, A.H. (2006). Studies on spasmogenic and spasmolytic activities of *calendula officinalis* flowers. *Phytother Research*, 20: 206-910.

Bharnagar, S.; Dixit, V.K.; Nigam, S.S.; Verma, K.C. (1977). Antimicrobial activity of essential oils of leaves of *Blumea lacera* D.C. and *Blumea lanciniata* D.C. *Indian J. Hosp. Pharm*. XIV(L): 14-16.

Bhuvaneswari, C.; Kiranmayee, R.; Suryakala, G. and Archana, G. (2013). Improved gymnemic acid production in the suspension cultures of *Gymnema sylvestre* through biotic elicitation. *Plant Biotechnology Reports*. DOI 10.1007/s11816-013-0290-3.

Boardley, M.; Stirton, C.H. and Harborne, J.B. (1986). A chemotaxonomic survey of the tribe Psoralea in Africa. *Biochem. Syst. Ecol*. 14 (6): 603–613.

Bohlmann, F. and Zdero, C. (1969). On a new coniferyl alcohol derivative from *Blumea lacera D. Tetrahed. Lett*. 2: 69-70.

Boulos, L. (2009). Flora of Egypt cheklist. Revised Annonated Edition, Alhadara Publishing, Egypt.

Bowen, I.H.; Cubbin, I.J. and Macleod, R.D. (1988). Furoquinoline alkaloids of tissue cultures of *Ruta graveolens*. *Of pharmacy and pharmacog*, 40**:** supplement, 107 p.

Calabrese, C.; Berman, S.H.; Babish, J.G.; Ma, X.; Shinto, L.; Dorr, M.; Wells, K.; Wenner, C.A. and Standish, L.J. (2000). A phase I trial of andrographolide in HIV positive patients and normal volunteers. Bastyr University Research Institute, Bastyr University, Washington 98028, USA. *Phytother Res.,* 14: 333–338.

Chanda, S.; Kaneria, M. and Nair, R. (2011). Antibacterial activity of *Psoralea corylifolia* L. seed and aerial parts with various extraction methods. *Res. J. Microbiol.* 60(2): 124–131.

Chaturvedi, H.C.; Jain, M. and Kidwai, N.R. (2007). Cloning of medicinal plants through tissue culture. a review. *Indian J Exp Biol*., 45:937–948.

Chebouat, E.; Dadamoussa, B.; Gharabli, S.; Gherraf, N.; Allaoui, M.; Cheriti, A.; Lahham, A. and Zellagui, A. (2014). Assessment of antimicrobial activity of flavonoids extract from *Ephedra alata*. *Der Pharmacia Lett*. 6(3): 27–30.

Chen, X.; Mou, Y.; Ling, J.; Wang, N.; Wang, X. and Hu, J. (2015). Cyclic dipeptides produced by fungus *Eupenicillium brefeldianum* HMP-F96 induced extra cellular alkalinization and H_2O_2 production in tobacco cell suspensions. *World J Microbiol Biotechnol*, 31(1): 247–253.

Chiu, C.S.; Chiu, Y.J.; Wu, L.Y.; Lu, T.C.; Huang, T.H.; Hsieh, M.T.; Lu, C.Y.; Peng, W.H. (2011). Diosgenin ameliorates cognition deficit and attenuates oxidative damage in senescent mice induced by D-galactose. *Am J Chin Med.,* 39:551–563.

Cinatl, J.; Morgenstern, B.; Bauer, G.; Chandra, P.; Rabenau, H. and Doerr, H. (2003). Glycyrrhizin, an active component of licoriceroots, and replication of SARS-associated corona virus. *Lancet*, 361: 2045–2046.

Cordell, G.A. (1997). The Alkaloids: Chemistry and Biology. Academic Press, New York.

Devi, C.S. and Srinivasan, V.M. (2011). *In vitro* studies on stimulation of Gymnemic acid using fungal elicitor in suspension and bioreactor based cell cultures of *Gymnema sylvestre* R. BR. *Recent Research in Science and Technology*, 3(4): 101-104.

Dangi, R.; Misar, A.; Tamhankar, S. and Rao, S. (2014). Diosgenin content in some *Trigonella* species. *Indian J Adv Plant Res*., 1:47–51.

Deshpande, H.A. and Bhalsing, S.R. (2014). Isolation and characterization of diosgenin from in vitro cultured tissues of *Helicteres isora* L. *Physiol Mol Biol Plants,* 20(1):89–94.

Deshpande, H.A. and Bhalsing, S.R. (2015). Plant derived novel biomedicinal: diosgenin. *Int J Pharmacog Phytochem Res.,* 6: 780–784.

Dixit, V.K. and Verma, K.C. (1976). Effect of essential oil of leaves of *Blumea lacera* DC on central nervous system. *Ind. J. Pharmac*. 8(1): 7-11.

Elissawy, A.M.; Ebada, S.S.; Ashour, M.L.; El-Neketi, M.; Ebrahim, W. and Singab, A.B. (2019). New Secondary Metabolites from the Mangrove-Derived Fungus Aspergillus Sp. AV-2. *Phytochemistry Lett.* 29: 1–5. doi:10.1016/ j.phytol.2018.10.014

Fang, W.; Lin, X.; Wang, J.; Liu, Y.; Tao, H.; and Zhou, X. (2016) Asperpyrone-Type Bis-Naphtho-γ-Pyrones with COX-2-Inhibitory Activities from Marine- Derived Fungus *Aspergillus niger*. *Molecules,* 21(7): 941. doi:10.3390/ molecules21070941.

Frank, S.; Caffieri, S.; Raffaelli, A.; Vedaldi, D. and Dall'Acqua, F. (1998). Characterization of psoralen-oleic acid cyclo adducts and their possible involvement in membrane photo damage. *J. photochem. Photobiol. B*. 44 (1): 39–44.

Gaid, M.M.; Scharnhop, H.; Ramadan, H.; Beuerle, T. and Beerhues, L. (2011). 4-Coumarate: CoA ligase family members from elicitortreated *Sorbus aucuparia* cell cultures. *J. Plant Physiol*. 168 (9): 944–951.

Gadzovska S.S.; Tusevski O.; Maury S.; Hano C.; Delaunay A.;Chabbert B.; Lamblin F.; Lainé E.; Joseph C. and Hagège, D. (2015). Fungal elicitor-mediated enhancement in phenylpropanoids and naphto-dianthrone contents of *Hypericum perforatum* L. cell cultures. *Plant Cell Tissue Organ. Cult*. 122: 213–226. DOI: http://doi.org/10.1007/s11240-015-0762-y.

Ghada, A.; Hegazi, H.; Ghareb, E. and Gabr, M.F. (2020). Ephedrine production from suspension cultures of *Ephedra alata* L. *Journal of Biotechnology, Computational Biology and Bionanotechnology*, 101(1): 25–33 C.

Gibbons, J.G. and Rokas, A. (2013). The function and evolution of the *Aspergillus* genome. *Trends in Microbiology*. 1: 14-22.

Godfrey, T. and West, S. (1996). Introduction to industrial enzymology. Industrial enzymology, *Mac. Millan Press, London*, 1-8.

Gupta, M.; Mazumder, U.K.; Thamilselvan, V.; Manikandan, L.; Senthilkumar, G.P.; Suresh, R. *et al*. (2007). Potential hepatoprotective effect and antioxidant role of methanol extract of *Oldenlandia umbellata* in carbon tetrachloride induced hepatotoxicity in Wistar rats. *Iran J. Pharmacol. Ther*. 6: 5-9.

Hao, S.; Xu, R.; Li, D.; Zhu, Z.; Wang, T. and Liu, K. (2015). Attenuation of streptozotocin-induced lipid profile anomalies in the heart, brain, and mRNA expression of HMG-CoA reductase by diosgenin in rats. *Cell Biochem Biophys,* 72(3): 741–749.

Harkes, P.; Krijbolder, L.; Libbenga, K.; Wijnsma, R.; Nsengiyaremge, T. and Verpoorte, R. (1985). Influence of various media constituents on the growth of *Cinchona ledgeriana* tissue cultures and the production of alkaloids and anthraquinones. *Plant Cell Tissue Organ Cult*. 4: 199-214.

Hiort, J.; Maksimenka, K.; Reichert, M.; Perovi´c-Ottstadt, S.; Lin, W.H.; Wray, V.*; et al*. (2004) New Natural Products from the Sponge-Derived Fungus *Aspergillus niger*. *J. Nat. Prod*. 67(9): 1532–1543. doi:10.1021/ np030551d.

Hiraoka, N.; Tashimo, K.; Kinoshita, C. and Hiro,oka M. (1996). Genotype and alkaloid contents of *Datura metal* varieties, *Biol Pharma Bull*, 19: 1086-1089.

Hostettmann, K. and Marston, A. (2005). Saponins. Cambridge University Press, Cambridge.

Hussain, M.S.; Fareed, S.; Saba Ansari, M.; Rahman, A.; Ahmad, I.Z.; Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. *J Pharm Bioallied Sci.,* 4(1): 10.

Ibrahim, M.M.; Rabab, M.A. and Farah, Q.A. (2019). Influence of biotic elicitor *Aspergillus niger* on salicylic acid products in callus culture of *Calendula officinalis* L. *Plant.J. of Physics*, 1294.

Ito, M.; Nakashima, H.; Baba, M.; Pauwels, R.; De Clercq, E.; Shigeta, S. and Yamamoto, N. (1987). Inhibitory effect of glycyrrhizin on the *in vitro* infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)]. *Antivir Res*., 7:127–137.

Ito, M.; Sato, A.; Hirabayashi, K.; Tanabe, F.; Shigeta, S.; Baba, M.; De Clercq, E.; Nakashima, H. and Yamamoto, N. (1988). Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). *Antivir Res* 10: 289–298.

Jaradat, N.; Hussen, F. and Al-Ali, A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. *J. Mater. Environ. Sci*., 6(6): 1771–1778.

Jatav, V.S.; Singh, S.K.; Khatri, P. and Sharma, A.K. (2011). Recent pharmacological trends of *Glycyrrhiza glabra* Linn. *Unani Res*., 1:1–11.

Jayachandran, K.S.; Vasanthi, H.R. and Rajamanickam, G.V. (2009). Anti-lipoperoxidative and membrane stabilizing effect of diosgenin, in experimentally induced myocardial infarction. *Mol Cell Biochem*., 327: 203–210.

Karwasara, V.S.; Jain, R.; Tomar, P. and Dixit, V.K. (2010). Genetic transformation and elicitation as yield enhancement strategy for glycyrrhizin production by cell cultures of *Abrus precatorius* L. in vitro. *Cell. Dev. Biol. Plant*. 46: 354–362.

Karwasara, V.S.; Tomar, P. and Dixit, V.K. (2011). Influence of fungal elicitation on glycyrrhizin production in transformed cell cultures of *Abrus precatorius* L. *Pharmacogn Mag*. 7(28): 307–313.

Kaur, T.; Singh, B.; Kaur, A. and Kaur, S. (2015). Endophyte-mediated Interactions between Cauliflower, the *Herbivore Spodoptera* Litura, and the *Ectoparasitoid BraconHebetor*. *Oecologia* 179(2): 487–494. doi:10.1007/s00442-015-3358-7.

Khan, A.; Jan, G.; Khan, A.; Jan, F.G.; Bahadur, A. and Danish, M. (2017). *In vitro* antioxidant and antimicrobial activities of *Ephedra gerardiana* (root and stem) crude extract and fractions. Evidence-based Complementary and Alternative Medicine, Article ID4040254.

Khushboo, P.S.; Jadhav, V.M.; Kadam, V.J. and Sathe, N.S. (2010). *Psoralea corylifolia* Linn.-‘‘Kushtanashini’’. *Pharmacogn Rev*. 4(7): 69–76.

Knopp, E.; Strauss, A. and Wehrli, W. (1988). Root induction on several *Solanaceae* species by *Agrobacterium rhizogenes* and the determination of root tropane alkaloid content, *Plant cell Rep*, 7: 590-593.

Kumar, R.A.; Sridevi, K.; Kumar, N.V.; Nanduri, S. and Rajagopal, S. (2004). Anticancer and immuno-stimulatory compounds from *Andrographis paniculata*. J Ethnopharmacol., 92: 291–295.

Kumar, V.; Desai, D. and Shriram, V. (2014). Hairy root induction in *Helicteres isora* L. and production of diosgenin in hairy roots. *Nat Prod Bioprospect,* 4: 107–112. https://doi.org/10.1007/ s13659-014-0011-9.

Laakso, I.; Seppanen-Laakso, T.; Hiltunen, R. and Ekundayo, O. (1989). Composition of the essential oil of *Blumea lacera* DC. Asteraceae leaves from Nigeria. *Flav. Fragr. J*. 4: 73-76.

Ajungla, L.; Patil, P.P.; Barmukh, B. and Nikam, T.D. (2009). Influence of biotic and abiotic elicitors on accumulation of hyoscyamine and scopolamine in root cultures of *Datura metel* L. *Indian Journal of Biotechnology*, 8: 317-322.

Li, D.-H.; Han, T.; Guan, L.-P.; Bai, J.; Zhao, N.; Li, Z.-L.; *et al*. (2016) New Naphthopyrones from marine-derived Fungus *Aspergillus niger* 2HL-M-8 and Their *In Vitro* Antiproliferative Activity. *Nat. Product. Res*. 30(10): 1116–1122. doi:10.1080/14786419.2015.1043553

Limberger, R.P.; Jacques A.L.B.; Schmitt G.C. and Arbo, M.D. (2013). Pharmacological effects of Ephedrine. [in:] Natural Products. Ed. Ramawat K.G.; Merillon J.M. Springer-Verlag, *Berlin, Heidelberg*: 1218–1233. DOI: http://doi.org/10.1007/978-3-642-22144-6_41.

Li, P.; Mou, Y.; Lu, S.; Sun, W.; Lou, J.; Yin, C. and Zhou, L. (2012). Quantitative determination of diosgenin in *Dioscorea zingiberensis* cell cultures by microplate-spectrophotometry and high-performance liquid chromatography. *Afr J Pharm Pharmacol,* 6: 1186–1193.

Liu, D.; Li, X.-M.; Li, C.-S.; and Wang, B.-G. (2013) Nigerasterols A and B, Antiproliferative Sterols from the Mangrove-Derived Endophytic Fungus *Aspergillus niger* MA-132. Hca 96 (6), 1055–1061. doi:10.1002/ hlca.201200332.

Liu, H.M.; Kiuchi, F. and Tsuda, Y. (1992). Isolation and structure elucidation of gymnemic acids, anti-sweet principles of *Gymnema sylvestre*. *Chem Pharm Bull.,* 40: 1366–1375.

Liu, W.K.; Xu, S.X. and Che, C.T. (2000) Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. *Life Sci*. 67: 1297-1306.

Liu, Y.; Nan, L.; Liu, J.; Yan, H.; Zhang, D. and Han, X. (2016). Isolation and Identification of Resveratrol-Producing Endophytes from Wine Grape Cabernet Sauvignon. *Springerplus* 5. doi:10.1186/s40064-016-2571-0.

Manganyi, M.C.; Regnier, T.; Kumar, A.; Bezuidenhout, C.C. and Ateba, C.N. (2018). Biodiversity and Antibacterial Screening of Endophytic Fungi Isolated from *Pelargonium Sidoides*. *South Afr. J. Bot*. 116: 192–199. doi:10.1016/ j.sajb.2018.03.016.

Manni, P.E. and Sinsheimer, J.E. (1965). Constituents from *Gymnema sylvestre* leaves. *J Pharm Sci.,* 54(10):1541–1544. doi:10.1002/ jps.2600541035.

Manjula, R. and Mythili, T. (2012). Improved phytochemical production using biotic and abiotic elicitors in *Marsilea quadrifolia. International Journal Current Science*, 98-101.

Mathur, M. (2018). Effect of fungal elicitation on growth andmetabolite production in callus of few medicinal plants. *Inter. J. Life Sci*. A9: 114–116.

May, G. and Adams, T. (1997). The Importance of Fungi to Man. *Genome Research*, 7: 1041-1044.

Misal, D.R.; Kharde, A.V.; Deshmukh, V.D. and Talekar, B.K. (2020). Elicitation of bacoside content using *Aspergillus niger* filtrate in *Bacopa monnieri* (Brahmi). *Journal of Pharmacognosy and Phytochemistry*, 9(5): 3311-3313.

Moinuddin, M.A. Vakil, and Vijay, D.M. (2013). Enhanced synthesis of andrographolide by *Aspergillus niger* and

Penicillium expansum elicitors in cell suspension culture of *Andrographis paniculata* (Burm. f.) Nees. *Vakil and Mendhulkar Botanical Studies*, 54: 49.

Mulabagal, V. and Tsay, H.S. (2004). Plant cell cultures-an alternative and efficient source for the production of biologically important secondary metabolites. *Int J Appl Sci Eng.,* 2(1): 29–48.

Muthukumar, B.; Arochiasamy, D.I. and Natarajan, E. (2004). Direct organogenesis in *Datura metel* L. from *in vitro* and *in vivo* nodal explants, *Indian J. Biotechnol*, 3: 449-451.

Namdeo, A.G. (2007). Plant cell elicitation for production of secondary metabolites: A review. *Pharmacognosy reviews*, 1(1): 69-79.

Nasrollahi, V.; Mirzaie-asl, A.; Piri, K.; Nazeri, S.; Mehrabi, R. (2014). The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in licorice (*Glycyrrhiza glabra*). *Phytochemistry*, 103: 32–37.

Ozkan, I.; Kose, O.; Ozmen, I. and Arca, E. (2012). Efficacy and safety of non-laser, targeted UVB phototherapy alone and in combination with psoralen gel or calcipotriol ointment in the treatment of localized, chronic, plaque-type psoriasis. *Int. J. Dermatol*. 51(5): 609–613.

Pal, R.; Moitra, S.K.; Chakravarti, N.N.; Adhya, R.N. (1972). Campesterol from *Blumea lacera* DC. *Phytochem*. 11, 1855.

Parijat, K.; Rekha, S. and Madhusudan, K. (2007). *Gymnema sylvestre*: a memoir. *J Clin Biochem Nutr.,* 41(2): 77–81. doi:10.3164/jcbn. 2007010.

Parsaeimehr, A.; Sargsyan, E. and Javidnia, K. (2010). A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of *Ephedra*. *Molecules*, 15(3): 1668–1678.

Patel, K.; Gadewar, M.; Tahilyani, V. and Patel, D.K. (2012). A review on pharmacological and analytical aspects of diosgenin: a concise report. *Nat Prod Bioprospect,* 2: 46–52.

Perrone, G.; Stea, G.; Epifani, F.; Varga, J.; Frisvad, J.C. and Samson, R.A. (2011). *Aspergillus niger* Contains the Cryptic Phylogenetic Species A. Awamori. *Fungal Biol*., 115(11): 1138–1150. doi:10.1016/j.funbio. 2011.07.008.

Pitta-Alvarez, S.I.; Spollansky, T.C. and Giulietti, A.M. (2000). The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia Candida*, *Enzyme Microb Technol,* 26: 252-258.

Poutaraud, A.; Bougrgaud, F.; Girardin, P. and Gotier, E. (2000). Cultivation of *Ruta graveolens* for the production of furanocoumarins of the therapeutic value. *Can. J. Bot*., 78: 1326-1335.

Quisumbing, E. (1998). Medicinal Plants of the Philippines, Katha Publishing Co. pp.966-967.

Ragasa, C.Y.; Wong, J. and Rideout, J.A. (2007). Monoterpene glycoside and flavonoids from *Blumea lacera*. *J. Natural Med*. 61(4): 474-475.

Rahimi, S.; Kim, Y.J. and Yang, D.C. (2015). Production of ginseng saponins: elicitation strategy and signal transductions. *Applied Microbiology and Biotechnology*. 2099(17): 6987-6996.

Rao, C.B.; Rao, T.N. and Muralikrishna, B. (1997). Flavonoids from *Blumea lacera*. *Planta Med*. 31: 235-237.

Rao, S.R. and Ravishankar, G.A. (2002). Plant cell cultures: chemical factories of secondary metabolites. *Biotechnology Advances*. 20(2): 101-153.

Rekha, S.; Srinivasan, V.; Saradha, V. and Hamsaveni, G.R. (2006). The *in vitro* antibacterial activity of *Hedyotis umbellata*. *Indian J. Pharm. Sci*. 68: 236-238.

Radman, R.; Saez, T.; Bucke, C. and Keshavarz, T. (2003). Elicitation of plant and microbial systems. *Biotechnol Appl Biochem*., 37: 91-102.

Samrin, S.; Varsha, S.; Tushar, K. and Vinay, K. (2020). Biotic elicitors enhance diosgenin production in *Helicteres isora* L. suspension cultures via up-regulation of CAS and HMGR genes. *Physiol Mol Biol Plants March*, 26(3): 593–604. https://doi.org/10.1007 /s12298-020-00774-6.

Saranya, H. and Velayutham, P. (2019) Effect of Fungal Elicitors on Plant Growth in the *in vitro* Cultures of *Oldenlandia umbellata* L. *Adalya Journal* Volome 8, Issue 12, December.

Schuster, E.; Dunn-Coleman, N.; Frisvad, J.; van Dijck, P. (2002). On the safety of *Aspergillus niger* – a review. *Applied Microbiology and Biotechnology*. 59: 426-435.

Selim, S. and Al-Jaouni, S. (2015). Anticancer and apoptotic effects on cell proliferation of diosgenin isolated from *Costus speciosus* (Koen.) Sm. *BMC Compl Altern Med.,* 15: 301.

Sethi, G.; Shanmugam, M.; Warrier, S.; Merarchi, M.; Arfuso, F.; Kumar, A.; Bishayee, A. (2018). Pro-apoptotic and anti-cancer properties of diosgenin: a comprehensive and critical review. *Nutrients,* 10:645.

Seydel, P. and Dornenburg, H. (2006). Establishment of *in vitro* plants, cell and tissuecultures from *Oldenlandia affinis* for the production of cyclic peptides. *Plant Cell Tiss. Org. Cult*. 85: 247-255.

Shanker, A.K. and Shanker, C. (2016). A biotic and biotic stress in plant. Recent advances and future perspectives. In Technology open, India, 527-563.

Shibata, S. (2000). A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. *J Pharm Soc Japan,* 120: 849–862.

Shreelalitha, S.J. and Sridhar, K.R. (2015). Endophytic Fungi of Wild Legume *Sesbania Bispinosa* in Coastal Sand Dunes and Mangroves of the Southwest Coast of India. *J. For. Res*. 26(4): 1003–1011. doi:10.1007/s11676-015-0103-3.

Singh, S.M.; Yadav, L.S.; Singh, S.K.; Singh, P.; Singh, P.N. and Ravindra, R. (2011). Phosphate Solubilizing Ability of Two Arctic *Aspergillus niger* Strains. *Polar Res*. 30. doi:10.3402/polar.v30i0.7283.

Siva, R. (2007). Status of natural dyes and dye yielding plants in India. *Curr. Sci.* 92: 916-925.

Siva, R.; Mayes, S.; Behera, S.K. and Rajasekaran, C. (2012). Anthraquinone dye production using root cultures of *Oldenlandia umbellata* L. *Ind Crop Prod*. 37: 415-419.

Siva, R.; Mudgal, G.; Rajesh, D.; Khan, F.N.; Vijyakumar, V. and Rajasekaran, C. (2009). Chara- cterization of novel pH indicator of natural dye *Oldenlandia umbellata* L. *Nat. Prod. Res*., 23: 1210-1217.

Soltan, M.M. and Zaki, A.K. (2009). Antiviral screening of forty-two Egyptian medical plants. *J. Ethnopharmacol.* 126(1): 102–107.

Soltani, J. and Moghaddam, M.S.H. (2014). Diverse and Bioactive Endophytic Aspergilli Inhabit Cupressaceae Plant Family. *Arch. Microbiol.* 196(9): 635–644 doi:10.1007/s00203-014-0997-8

Srinivasan, S. and Sarada, D.V. (2012). Antifungal activity of phenyl derivative of Pyranocoumarin from *Psoralea corylifolia* L. Seeds by inhibition of acetylation activity of trichothecene 3-O-acetyltransferase (Tri101). *J. Biomed. Biotechnol.* 310850.

Syed, A.A.; Mirza, M.V.B. (2014). Biotic elicitor enhanced production of psoralen in suspension cultures of *Psoralea corylifolia* L. *Saudi Journal of Biological Sciences,* 21: 499–504.

Szliszka, E.; Czuba, Z.P.; Se˛dek, Paradysz, A.; Krol, W. (2011). Enhanced TRAIL-mediated apoptosis in prostate cancer cells by the bioactive compounds neobavaisoflavone and psoralidin isolated from *Psoralea corylifolia*. *Pharmacol. Rep*. 63(1): 139–148.

Taha, H.S. (2003). Effect of Biotic Stress (*Aspergillus niger*) on the Production and Accumulation of Total Alkaloids in *Atropa belladonna* L. via Tissue Culture Proc. Int. Conf. on MAP Eds. J. Bernáth et al. Acta Hort. 597, ISHS.

Taha, H.S.; El-Bahr, M.K.; Seif-El-Nasr, M.M. (2009). In vitro studies on Egyptian *Catharanthus roseus* (L.). II. Effect ofbiotic and abiotic stress on indole alkaloids production. *J.Appl. Sci. Res*., 5: 1826–1831.

Takahashi, K.; Inoue, H.; Sakai, K.; Kohama, T.; Kitahara, S.; Takishima, K.; Tanji, M.; Athauda, S.; Takahashi, T. and Akanuma, H. (1991). The primary structure of *Aspergillus niger* acid proteinase A. *The Journal of Biological Chemistry*. 266: 19480-19483.

Takano, D.; Nagamitsu, T.; Ui, H.; Shiomi, K.; Yamaguchi, Y.; Masuma, R.; *et al*. (2001). Absolute Configuration of Nafuredin, a New Specific NADH-Fumarate Reductase Inhibitor. *Tetrahedron Lett*. 42(16): 3017–3020. doi:10.1016/s0040-4039(01)00355-0.

Talfan, A.A.; Al-Mahdawe, M.M. and Nadir, D.S. (2020). Influence of the extract of biotic elicitor *Aspergillus niger* on the production of furanocoumarins in callus cultures of *Ruta graveolens Plant Archives,* 20(2): 4633-4638.

Thakur, G.S.; Sharma, R.; Sanodiya, B.S.; Baghel, R.; Thakur, R.; Singh, B.N. (2013). *In vitro* induction of tuber formation for the synthesis of secondary metabolites in *Chlorophytum borivilianum* Sant. et Fernand. *African journal of Biotechnology*, 2(20).

Toghueo, R.M.K.; Sahal, D.; Zabalgogeazcoa, Í.; Baker, B. and Boyom, F.F. (2018). Conditioned media and Organic Elicitors Underpin the Production of Potent Antiplasmodial Metabolites by Endophytic Fungi from Cameroonian Medicinal Plants. *Parasitol. Res*. 117(8): 2473–2485. doi:10.1007/s00436-018-5936-1.

Uchoa, P.K.S.; Pimenta, A.T.A.; Braz-Filho, R.; de Oliveira, M.d.C.F.; Saraiva, N. N.; Rodrigues, B.S.F. (2017). New Cytotoxic Furan from the marine Sediment-Derived Fungi *Aspergillus niger*. *Nat. Product. Res.* 31(22): 2599–2603. doi:10.1080/14786419. 2017. 1283499.

Mendhulkar, V.D.; Patade, P. and Vakil, M. (2016). Elicitation of Flavonoids in *Blumea lacera* (Burm.f.) DC. Cell Culture using Chemical Elicitor, Salicylic Acid and Biological Elicitor, *Aspergillus niger Int. J. Curr. Res. Biosci. Plant Biol.* 3(11): 85-91. doi: http://dx.doi.org/10.20546/ijcrbp.2016.311.013.

Wang, *J. et al*. (2015). Enhanced production of flavonoids by methyl jasmonate elicitation in cell suspension culture of *Hypericum perforatum*. *Bioresour Bioprocess*; 2: 1–9.

Wang, L.-X.; Ren, L.-L.; Liu, X.-B.; Shi, J.; Wang, J.-Z. and Luo, Y.-Q. (2019) Effects of Endophytic Fungi in Mongolian pine on the Selection Behavior of Woodwasp (*Sirex noctilio*) and the Growth of its Fungal Symbiont. *Pest Manag. Sci.* 75(2): 492–505. doi:10.1002/ps.5146.

Wang, Y.J.; Pan, K.L.; Hsieh, T.C.; Chang, T.Y.; Lin, W.H. and Hsu, J.T. (2011). Diosgenin, a plant-derived sapogenin, exhibits antiviral activity in vitro against hepatitis C virus. *J Nat Prod.,* 74(4): 580–584.

Wang, Y.; Ye, X.; Ma, Z.; Liang, Q.; Lu, B.; Tan, H.; Xiao, C.; Zhang, B. and Gao, Y. (2008). Induction of cytochrome P450 1A1 expression by ginsenoside Rg1 and Rb1 in HepG2 cells. *Eur. J. Pharmacol*. 601: 73-78.

Warrier, P.K.; Nambiar, V.P.K. and Ramankutty, C. (1996). Indian Medicinal Plants. Arya Vaidya Sala, Kottakkal. *Orient Longman Press*. pp.135-138.

Wen, N. and Ri-qiang, C. (1996). Fractionation and biological activity of *Aspergillus oryzae* elicitor promoting biosynthesis of shikonin derivatives. *Acta Botanica Sinica,* 38(5): 367–374.

Wessner, D.; Hofmann, H. and Ring, J. (1999). Phytophotodermatitis due to *Ruta graveolens* applied as protection against evil spells. *Contact Dermatitis*, **41:** 232.

Wiktorowska, E.; Dugosz, M.; Janiszowska, W. (2010). Significant enhancement of oleanolic acid accumulation by biotic elicitors in cell suspension cultures of *Calendula officinalis*L. *Enzyme Microb. Technol*. 46(1): 14–20.

Xie, H.; Zhuang, X.; Bai, Z.; Qi, H.; and Zhang, H. (2006). Isolation of Levoglucosan-Assimilating Micro-organisms from Soil and an Investigation of their Levoglucosan Kinases. *World J. Microbiol. Biotechnol*. 22(9): 887–892. doi:10.1007/s11274-006-9133-5.

Xu, M-J *et al*. (2005). Nitric oxide mediates the fungal elicitor-induced hypericin production of *Hypericum perforatum* cell suspension cultures through a jasmonic-acid-dependent signal pathway. *Plant Physiol*. 139: 991–998.

Yan, Q.; Zhou, X.-W.; Zhou, W.; Li, X.-W.; Feng, M.-Q. and Zhou, P. (2008). Purification and properties of a novel ß-glucosidase, hydrolyzing ginsenoside Rb1 to CK, from *Paecilomyces Bainier*. *J. Microbiol. Biotechnol*. 18: 1081-1089.

Yari Khosroushahi, A.; Valizadeh, M.; Ghasempour, A.; Khosrowshahli, M.; Naghdibadi, H.; Dadpour, M. *et al*. (2006). Improved Taxol production by combination of inducing factors in suspension cell culture of *Taxus baccata*. *Cell Biol. Intl*. 30(3): 262–269.

Youl Her, Young-Chul Lee, Jin-Hwan Oh, Yoon-E Choi, Chang-Woo Lee, Jin-Suk Kim, Hwan Mook Kim, and Ji-Won Yang (2012). An Application of *β*-glycosidase to Transformation of Ginsenosides for the Effective Production of Specific Ginsenosides with Biological

Efficacy. *Biotechnology and Bioprocess Engineering* 17: 538-546 DOI 10.1007/s12257-011-0678-2.

Yu, H.; Zhang, C.; Lu, M.; Sun, F.; Fu, Y. and Jin, F. (2007). Purification and characterization of new special ginsenosidase hydrolyzing multi-glycisides of protopanaxadiol ginsenosides, ginsenosidase type I. *Chem. Pharm. Bull*. 55: 231-235.

Yue, W.; Ming, Q.; Lin, B.; Rahman, K. and Zheng, C. (2016). Medicinal plant cell suspension cultures: pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. *Critical Reviews in Biotechnology*, 36(2): 215-232.

Zhang, Y.; Tang, L.; An, X.; Fu, E. and Ma, C. (2009). Modification of cellulase and its application to extraction of diosgenin from *Dioscorea zingiberensis* CH Wright. *Biochem Eng J,* 47(1–3):80–86.

Zhao, H.Y. and Frang, W.Y. (1991). Antithrombotic effect of *Andrographis paniculata* Nees in preventing myocardial infarction. *Chi Med J (Engl)* 104: 770–775.