

## Review

# Phenolic compounds from medicinal plants as Natural anti-elastase products for the therapy of pulmonary emphysema

Rachid Kacem

Department of Biology, Faculty of Natural Sciences and Life, Ferhat Abbas University, Setif 19000, Algeria.

Accepted 23, July 2013

Medicinal plants have been used as alternative treatments to health problems. Most of the natural remedies are based on medicinal plants. Many reports have demonstrated that the extracts from medicinal plants such as *Nigella sativa* (L.) seeds extracts have anti-elastase activity; this is mainly due to the enrichment of the extracts with many bioactive molecules mainly phenolic compounds. Neutrophil serine proteases including human neutrophil elastase are involved in many inflammatory diseases, such as chronic obstructive pulmonary disease and emphysema. Since the current therapies for these diseases are inadequate and have numerous adverse effects, there is an acute need of potential alternative therapies. The natural protease inhibitors have received increasing attention as useful tools for potential utilization in pharmacology. This review is elucidating the most important natural phenolic substances that have been reported recently for their effectiveness as natural anti-elastase molecules and hence to the possibility of their use in the field of pharmaceuticals.

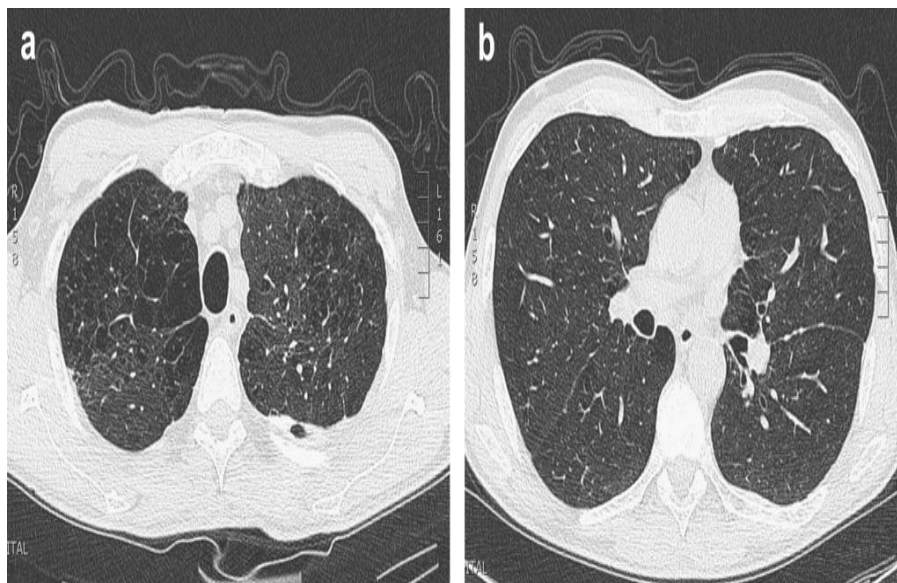
**Key words:** Medicinal plants, phenols, elastase, anti-elastase, chronic obstructive pulmonary disease (COPD), emphysema.

## INTRODUCTION

Over thousands of years, medicinal plants have been used as alternative treatments to health problems. An estimated 80% of the world's population depend largely on traditional natural medicine. Most of the natural remedies are based on medicinal plants, with some 35 000 plant species being used (WHO, 2008). Many research reports confirmed that the extracts from medicinal plants have anti-inflammatory activity; this is mainly due to the enrichment of the extracts with many bioactive molecules (Kacem, 2011). A large number of natural products have been used for the treatment of different kinds of diseases and continue to be one of the major sources of inspiration for drug discovery. In particular, these compounds are important in the treatment of life-threatening conditions (Grabley and Thiericke, 1999). Neutrophil serine proteases (NSPs) are involved in many inflammatory diseases, such as chronic

obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), emphysema and cystic fibrosis (CF). Among these enzymes, is neutrophil elastase (NE), an important granule enzyme that hydrolytically degrades extracellular matrix components such as elastin and proteoglycans (Owen et al., 1995).

Normally, the activity of NE that is released to the extracellular region is strictly regulated by endogenous macromolecular inhibitors, including  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI),  $\alpha_2$ -macroglobulin and secretory leuko-proteinase inhibitor (SLPI) (Korkmaz et al., 2008). However, in certain inflammatory states, a large number of neutrophils are infiltrated and activated by a stimulus such as platelet activating factor (PAF) and bacterial lipopolysaccharides. The abnormal exocytosis of NE results in a local imbalance of elastase-antielastase, which subsequently causes severe tissue damage



**Figure 1.** Computerized tomography scan of emphysema. Two slices from a patient with a 'heterogeneous' pattern of emphysema. (a) Slice through the apex of the lung showing emphysematous lung with numerous bullae and blebs in the right lung. (b) Slice through the mid-zone of the lung showing only mild emphysematous change (Nicola et al., 2012).

(Hiemstra et al., 2002). In the lungs, this inflammatory process results in various diseases including emphysema, ARDS, COPD, chronic bronchitis (CB), and CF (Greer et al., 2004). NPIs are widely distributed among plants, the major sources of PIs are storage organs such as seeds, these NPIs act as regulatory, defensive and storage proteins. The serine proteases inhibitors (SPIs) are the most studied and have been purified from various seeds (Araújo et al., 2005). The NPIs have received increasing attention as useful tools not only for the study of enzymes structure and reaction mechanisms, but also for potential utilization in pharmacology (Robert, 2005). NPIs continue to attract the attention of researchers due to their increasing use in medicine and biotechnology especially as powerful tools for inactivating target proteases in the pathogenic process of human diseases such as emphysema, arthritis, pancreatitis, thrombosis, cancer and acquired immune deficiency syndrome (AIDS). Due to the development of treatment complications, such as drug resistance and adverse effects, conventional medicine is still insufficient to provide a complete treatment of certain diseases (Ram et al., 2011); therefore, continuing research to discover new natural drugs is needed to provide alternative therapy. Natural compounds that directly inhibit NE or its release from human leukocytes are of great interest in the development of new anti-inflammatory drugs and therapies. Based on the importance of the use of natural products in this field, especially in the case of COPD and emphysema, and as long as there is no state of scientific publications reviewing what has been reported in the field of natural anti-elastase phenolic

products, it is important to develop a better understanding by providing an overview of the published data and elucidating the most important natural phenolic substances that have been reported recently for their effectiveness as natural anti-elastase molecules and hence to the possibility of their use in the field of pharmaceuticals.

#### **NEUTROPHIL ELASTASE AND PHYSIOPATHOLOGY OF EMPHYSEMA**

Emphysema refers to a long-term, progressive disease of the lungs that primarily causes shortness of breath. In patients with emphysema, the tissues necessary to support the physical shape and function of the lungs are destroyed (Figure 1). The destruction of lung tissue around the alveolis caused mainly by NSPs makes these air sacs unable to hold their functional shape upon exhalation. Most important risk factors that gave rise to emphysema are smoking, air pollution, age and protease-antiprotease imbalance (Willemse et al., 2004). In more common types of emphysema, usually there is a greater heterogeneity of disease with more units becoming poorly functional as disease progresses, resulting in a rise in residual volume and a fall in the vital capacity (VC). Emphysematous destruction results in loss of parallel airways and functional loss of patent airways supplying poorly ventilated areas in the lung (Barnes et al., 2003). Destruction of interstitial lung elastin is central to the pathogenesis of emphysema. Lung elastin is a long-lived protein in connective tissue and once destroyed by

elastolytic enzymes, emphysema will develop even though elastin may re-accumulate. Studies have shown that as neutrophils move in close proximity to connective tissue, they degrade it and these phenomena cannot be prevented by  $\alpha_1$ -AT (Stockley, 1999). This inability to prevent completely the connective tissue degradation is due to restricted access of inhibitors to the area. In  $\alpha_1$ -AT deficiency, the protease–antiprotease imbalance exists, whereas in subjects with normal  $\alpha_1$ -AT, the development of disease depends largely on the normal process of connective tissue degradation as cells migrate. There are many reports published in this issue describing the predominant pathogenic mechanism leading to emphysema, an imbalance between proteases especially elastolytic enzymes released in the lung (Raja, 2006). The imbalance favouring proteases and elastase leads to proteolytic damage to lung connective tissue, elastin degradation and emphysema (Stockley, 1999). The two main sources of elastase that causes such destruction in the lungs are neutrophils and pulmonary macrophages (Barnes et al., 2003). Animal studies have shown that when lung elastin is damaged by instillation of elastase into the lungs, the intact desmosine cross-links of elastin appear in the urine (Stone et al., 1990). Elastase is an enzyme that belongs to serine proteases family which comprises hydrolases that breakdown peptide bond. Once activated, the mature glycoprotein form is capable for the degradation of elastin in connective tissues by breaking down peptide bonds. Evidence for the involvement of NE in the pathophysiology of acute lung injury includes: (1) neutrophil elastase levels are increased in both clinical and animal models of acute lung injury; (2) many reports indicated that systemic administration of NE produces typical *in vivo* symptoms of acute lung injury and (3) inhibition of increased NE activity reduces symptoms of acute lung injury in animal models (Kawabata et al., 2002).

### SYNTHETIC ELASTASE INHIBITORS

Although several synthetic elastase inhibitors have been tested with great interest; there are few results reported in this field. Synthetic elastase inhibitors include small molecular weight synthetic compounds. Water soluble agents (approximately 1 mM or less) are generally ineffective in preventing emphysema, possibly because adequate amounts of the inhibitors are not in solution in the lungs (Snider et al., 1994). Irreversible inhibitors with high molecular weight, slowly clearing irreversible inhibitors such as recombinant  $\alpha_1$ -PI are effective in ameliorating emphysema for many hours in a dose dependent fashion. High molecular weight inhibitors, such as SLPI are tight binding and clear slowly from the lungs and prevent emphysema in a dose dependent fashion for many hours. Studies reported by Stone et al. (1990) demonstrated that emphysema was worsening

after using the reversible, rapidly clearing inhibitors, such as Boroval. This compound is a small molecular weight agent, high effective *in vitro* inhibitor (Stone et al., 1990). The intravenous route which is used with human  $\alpha_1$ -PI for augmentation therapy of severe  $\alpha_1$ -PI deficiency is not suitable, because of its expenses, discomfort and inconvenience, the ideal drug would be bio-available after oral administration, would appear in adequate concentration in the lungs when given orally, and would not interfere with the function of proteases elsewhere in the body. Aerosol administration was also found inconvenient, it has the drawback even with particles of the appropriate aerodynamic diameter and about 10% of an orally inhaled aerosol is deposited in the lungs. Thus, it might require long periods of inhalation each day to administer sufficient drug to alter the elastolytic burden of the lungs (Snider et al., 1994). Sivelestat is a selective NE inhibitor. A retrospective data analysis of septic patients associated with ARDS and disseminated intravascular coagulation was conducted to investigate the effects of sivelestat. It was possible from the results that sivelestat improves the outcome of septic patients associated with ARDS and disseminated intravascular coagulation. The analysis showed the sivelestat administration to be an independent predictor of survival of those patients (Hayakawa et al., 2010). The research work conducted by Vasconcelos et al. (2011) tested two new human neutrophil elastase (HNE) inhibitor peptides, which were synthesized based on the reactive-site loop of the Bowman-Bark inhibitor protein. The results indicated that these new peptides are competitive inhibitors for HNE and the inhibitory activity can be modulated by modifications introduced at the N- and C-terminal of the peptides. This study suggested their potential application in chronic wound treatment (Vasconcelos et al., 2011). The *in vitro* and *in vivo* studies conducted using chemically modified tetracycline-3 (CMT-3) demonstrated that among all tested CMTs, CMT-3 is the only CMT that can directly inhibit both the amidolytic activity of human leukocyte elastase (HLE). In addition, CMT-3 has been found to reduce leukocyte elastase activity *in vivo* in gingival extracts of rats with experimental periodontal disease. CMT-3 can inhibit pathologic connective tissue breakdown by two mechanisms: direct inhibition of neutral proteases (elastase and matrix metalloproteases (MMPs)); and protecting their endogenous inhibitors,  $\alpha_1$ -PI and TIMPs, from being digested and inactivated by MMPs and HLE, respectively (Gu et al., 2011).

### MEDICINAL PLANTS ARE SOURCES OF ANTI-ELASTASE PHENOLIC COMPOUNDS

Medicinal plants are considered to be excellent sources of phenolic compounds with very interesting nutritional and therapeutic applications. Extracts from plants have

been widely investigated and found to have anti-elastase activities. Several studies carried out in our laboratory indicated that *Nigella sativa* (L.) seeds and their purified constituents have been shown to have beneficial therapeutic potential for many diseases, such as COPD and emphysema. It has been reported in these studies that extracts from *N. sativa* (L.) seeds and its main constituents mainly monoterpenes inhibit HNE, and the highest inhibitory concentration caused total inhibition (Kacem, 2011). In one study, 150 plant extracts were tested for their ability to inhibit elastase in which six plants showed activity over 65%. These included cinnamon (*Cinnamomum cassia*), turmeric (*Curcuma longa*) and nutmeg (*Myristica fragrans*). Isolated green tea (*Camellia sinensis*) has been used to isolate polyphenols such as catechin and epigallocatechin gallate that have been found to be inhibitors of elastase (Kim et al., 2004). Frankincense (*Boswellia* species) resin was used to purify triterpenoids known as boswellic acids that have also been shown to have anti-elastase activity (Melzig et al., 2001). Polyphenols isolated from persimmon (*Diospyros kaki*) leaves showed anti-elastase activity (An et al., 2005). Using spectrophotometric analysis extracts from Rosemary (*Rosmarinus officinalis*) (Figure 1) have also been found to have inhibitory effects on elastase activity (Baylac and Racine, 1999). The natural products which have shown activity in these assays represent a wide variety of the types of phenolic compounds. From a panel of twenty three plant extracts, some exhibit high anti-elastase activities, these included white tea which was found to have very high phenolic content, along with high TEAC and SOD activities (Thring et al., 2009). In this recent study, the anti-elastase, anti-collagenase, and anti-superoxide activity of 21 plant extracts were determined in a chemical assay. Nine of the plant extracts exhibited anti-elastase activity, with the highest six being white tea (~89%), cleavers (~58%), burdock root (~51%), bladderwrack (~50%), anise and engelica (~32%). The phenolic content of the extracts varied between 0.05 and 0.26 mg gallic acid equivalent/ml with the exception of white tea, reported as 0.77 mg gallic acid equivalent/ml. White tea was clearly the superior plant extract in the study for anti-elastase activity. The influence of some essential oils, absolutes, resinoids, oleoresins and natural plant extracts were tested on the enzymatic activity of HLE (EC 3.4.21.37). Among them, poplar bud absolute, rosemary extract, benzoin resinoid and turmeric oleoresin (figure) had an inhibitory activity significantly higher than the reference, ursolic acid. Specifically, turmeric oleoresin was the most potent inhibitor of HLE. The observed inhibition by such absolutes, resinoids, oleoresins or plant extracts may encourage their use in aromatherapy (Thring et al., 2009). Fifteen extracts of different polarities (dichloromethane, methanol and aqueous extracts) from 5 medicinal plants (*Aspilia helianthoides*, *Ceropegia rupicola*, *Kniphofia sumarae*, *Pavetta longiflora*, *Plectranthus cf*

*barbatus*) were tested for their inhibitory effects against the activity of HNE (EC 3.4.21.37) among the plants tested, *A. helianthoides* was the most active inhibitor of HNE. The dichloromethane extract of this medicinal plant showed the most active inhibitory effect on the HNE activity ( $IC_{50} = 0.4 \mu\text{g/ml}$ ). The results provided some scientific justification for the use of *A. helianthoides* in traditional medicine and indicate the presence of HNE inhibitory constituents in the active tested plants, the authors reported that the observed HNE inhibitory effect of the tested extracts might be attributed to the phenolic compounds (Alasbahi and Melzig, 2008).

## PHENOLIC COMPOUNDS

Phenolic compounds are ubiquitous in plants which collectively synthesize several thousand different chemical structures characterized by hydroxylated aromatic ring(s), these compounds play several important functions in plants. They represent a striking example of metabolic plasticity enabling plants to adapt to changing biotic and abiotic environments and provide to plant products colour, taste, and putative health promoting benefits. Phenolic compounds represent the most studied phytochemicals and have been widely exploited as model systems in different areas of plant research (Newman and Cragg, 2007).

### Extraction, isolation and purification of phenolic compounds

Interest in the development of bioprocesses for the extraction of bioactive compounds from natural sources has increased in recent years due to the potential applications of these compounds in food, chemical, and pharmaceutical industries. Many methods have been developed to extract and produce phenolic compounds from medicinal plants, these include the following.

#### **Solid-state fermentation (SSF)**

SSF has received great attention, because this bioprocess has potential to successfully convert inexpensive agro-industrial residues, as well as plants, in a great variety of valuable compounds, including bioactive phenolic compounds. SSF has been defined as the fermentation process occurring in the absence or near-absence of free water. SSF processes generally employ a natural raw material as carbon and energy source. The amount of water absorbed could be several times more than its dry weight, which leads relatively high water activity on the solid/gas interface in order to allow higher rate of biochemical process. Low diffusion of nutrients and metabolites takes place in lower water activity conditions whereas compaction of substrate occurs at

higher water activity, only fungi and yeast were termed as suitable micro-organisms for SSF. In the early phases of SSF, temperature and concentration of oxygen remain uniform throughout the substrate, but as the fermentation progresses, oxygen transfer takes place resulting in the generation of heat. The transfer of heat out of SSF system is closely related with the aeration of fermentation system. The temperature of the substrate is also very critical in SSF as it ultimately affects the growth of the micro-organism, spore formation, germination, and product formation. SSF is a clean technology with great potential for application on the production or extraction of biologically active compounds from natural sources. The agro-industrial residues reuse in this area is of particular interest due to their availability, low cost, and characteristics that allow the obtaining of different bioactive phenolic compounds, besides being an environment friendly alternative for their disposal. Another interesting application for SSF is to increase the bioactive phenolic compounds content in food products. This area has great potential to expand in a near future due to the increased consumer desire to improve health through food (Martins et al., 2011).

#### **Microwave assisted extraction (MAE)**

MAE has been considered as a potential alternative to traditional solid-liquid extraction for the isolation of metabolites, such as phenolic compounds from plants. It has been chosen to extract such compounds for several reasons: (1) reduced extraction time, (2) reduced solvent usage, and (3) better extraction yield. Microwave assisted extraction of plant secondary metabolites may be affected by a large variety of factors, such as power and frequency of microwave, duration of microwave radiation, moisture content and particle size of plant samples, type and concentration of solvent, ratio of solid to liquid, extraction temperature, extraction pressure and number of extraction cycles. Of these factors, solvent is regarded as one of the most important parameters for microwave assisted extraction, which affects the solubility of the target components and the absorption of microwave energy determined by its dissipation factor. Furthermore, the amount of water present in solvent significantly influenced extraction yield (Chen et al., 2010).

#### **Supercritical fluid extraction (SFE)**

SFE is the process of separating one component from used as a sample preparation step for analytical another, using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be purposes, or on a larger scale to either strip unwanted material from a product or collect a desired product (e.g. essential oils). CO<sub>2</sub> is the most used supercritical fluid, extraction conditions for supercritical

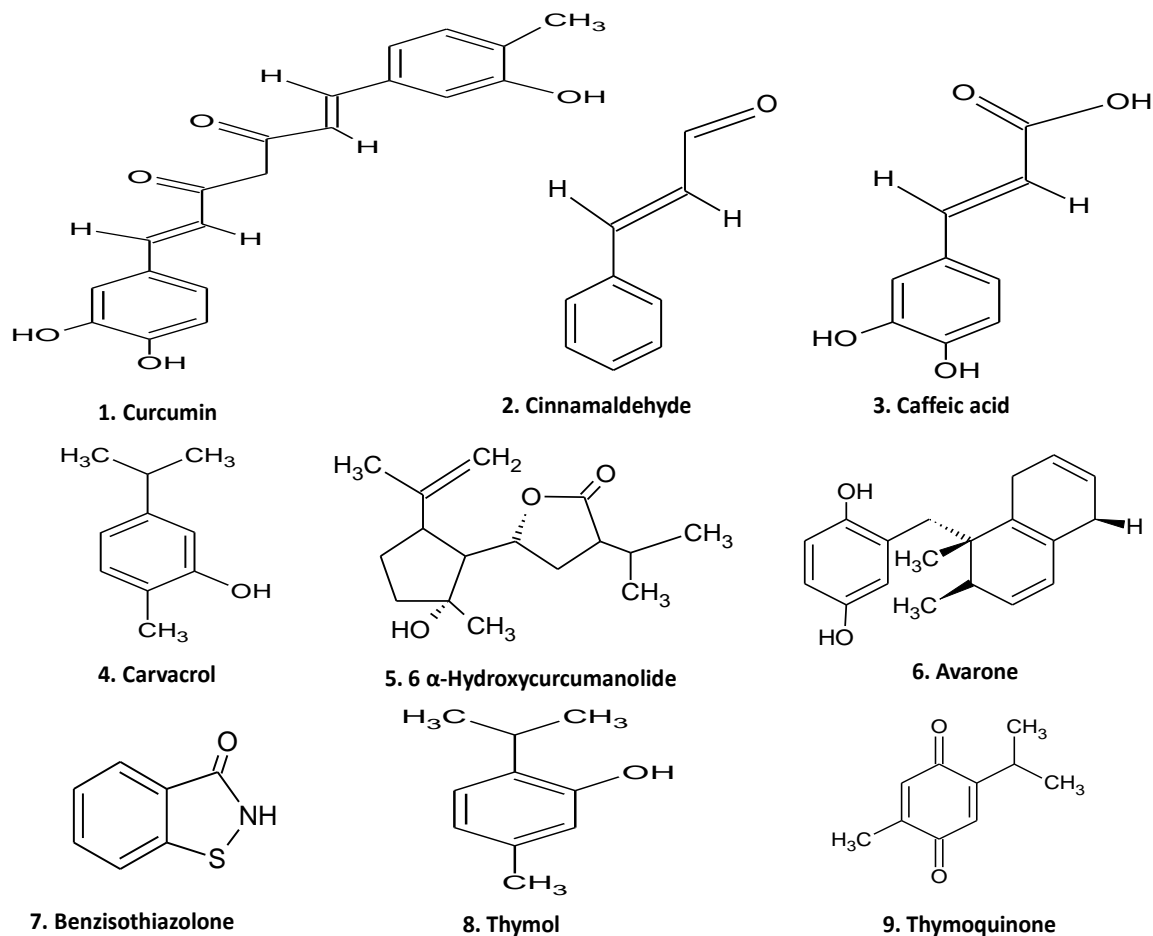
CO<sub>2</sub> are above the critical temperature of 31°C and critical pressure of 74 bars. Addition of modifiers may slightly alter this. SFE provides relatively clean extracts, free from certain degradation of labile or easily oxidized compounds which may emanate from lengthy exposure to high temperatures and oxygen, which can occur during the traditional extraction techniques (Vatai, 2009).

#### **Pressurized liquid extraction (PLE)**

PLE was developed for rapid and efficient extraction of analytes from solid matrices such as plant materials, extraction temperature is an important experimental factor, because elevated temperatures could lead to significant improvements in the capacity of extraction solvents to dissolve the analytes, in the rates of mass transport, and in the effectiveness of sample wetting and matrix penetration, all of which lead to overall improvement in the extraction and desorption of analytes from the surface and active sites of solid sample matrices. To achieve all these advantages, elevated pressure is needed to maintain the extraction solvents as liquids at high temperatures (usually above their boiling points). Although the PLE emerged in the mid 1990, it has rarely been applied to the extraction of plant materials (Benthin et al., 1999). In studies carried out using the PLE extracts from a selection of representative herbs were compared with extracts obtained according to pharmacopoeia monographs; their results indicated that PLE is often superior to other extraction methods currently used in crude herb analysis in terms of recovery, extraction time, and solvent consumption, the choice of extraction solvent is important factor in PLE. Most PLE applications employed the organic solvents commonly used in conventional techniques, e.g. methanol. Application of PLE reported by Kawamura et al. (1999) for the extraction of an active compound with significant medicinal interest, paclitaxel (commonly known as taxol, which has anticancer activity), from the bark of *Taxus cuspidate* indicated that the use of water alone as the extraction solvent is a viable alternative. An interesting result from this study was that although in conventional extraction methods, the taxol content of the water extract was very low and this was dramatically improved by use of the elevated temperature and pressure conditions of PLE.

#### **Detection of phenols**

The detection of phenolic compounds could be realized by application of different methods including CE; it is an analytical technique applicable for analyses of a great range of analytes, polar and charged compounds. The electrophoretic process is a differential movement of solutes caused by the attraction or repulsion in an electric field. The CE technique can replace the HPLC method



**Figure 2.** Structure of phenolic compounds showing inhibitory activity against HNE.

since it provides some advantages dealing with high efficiency, short analysis time, low solvent consumption, versatility and simplicity (Herrero and Mendiola, 2010). After carrying out the separation of the sample components, the detection of the analytes has to be made. Different detection systems can be coupled to CE; they can be classified in three groups: detection based on optical techniques (fluorescence, phosphorescence, UV-Vis absorption, chemiluminescence, infrared spectroscopy, NRM, Raman spectroscopy, refraction, etc); electrochemical techniques (such as conductometric, potentiometric, amperometric and voltametric detection); and other techniques like MS and radiochemical techniques. For obtaining a good separation in CE, it is necessary to optimise several parameters, such as buffer type, pH and concentration, type and dimensions of capillary, additives, temperature, voltage and injection mode. The influence of every parameter on the separation will be evaluated by the analyst and will depend on the CE methodology used, the kind of phenolic molecule under study and the matrix analysed (Herrero and Mendiola, 2010).

### Effects of phenolic compounds on HNE activity

Many studies have indicated that extracts from medicinal plants found to have very high phenolic content and their purified constituents mainly the phenols have been shown to have beneficial therapeutic potential for many diseases, such as COPD and emphysema (Kori et al., 2009). Recently, one of the well known plant derived compounds, curcumin (*C. longa*) (1; Figure 2) has been studied in C57BL/6 mice and was found to attenuate elastase and cigarette smoke induced pulmonary inflammation and emphysema. Furthermore, curcumin has not been reported for any side effect even at high doses and thus has been considered to be safe. Curcumin and triterpenoids have been found effective in experimental bronchitis and emphysema. Recent study conducted by Khan and Ahmed (2011) aimed to evaluate the effects of certain essential oils *Cinnamomum verum*, *Syzygium aromaticum*, *Cymbopogon citratus*, *Cymbopogon martini* and their major components cinnamaldehyde, eugenol, citral and geraniol, respectively. The results of this study indicated that more than 70% reduction in

elastase activity was observed by the oils of *C. verum*, *C. martini*, eugenol, cinnamaldehyde and geraniol. Maximum reduction (96.56%) in elastase activity was produced by cinnamaldehyde (2; Figure 2) (Khan and Ahmad, 2011). Many studies carried out to test the effect of caffeic acids (3; Figure 2) on elastase activity, all of these have indicated that these compounds are potent inhibitors of elastase, the recent study conducted by Dey et al. (2009) led to the isolation of two caffeic acid derivative esters, eicosanyl caffeate and docosyl caffeate. The two compounds exhibited potent elastase inhibitory activity, with  $IC_{50}$  values of 0.99 and 1.4  $\mu\text{g/ml}$ , respectively. The results indicate a possible role of caffeic acid derivatives, in addition to flavonoids in the anti-ulcer properties of *Glycyrrhiza glabra* (Dey et al., 2009). It has been reported that extracts from *N. sativa* (L.) seeds and its main constituents mainly monoterpenes inhibit HNE activity, and the highest inhibitory concentration caused total inhibition. Several research investigations have revealed that monoterpenes, mainly 5-isopropyl-2-methyl phenol (4; Figure 2) caused marked HNE inhibitory activity with very low  $IC_{50}$  values. The studies suggested that this compound could be considered as a natural anti-elastase agent and a possible candidate for phytotherapy, especially in the treatment of injuries that appear in some pathologic cases, such as COPD and emphysema (Kacem and Meraihi, 2006; Kacem, 2011). Studies carried out by Thring et al. (2009), have indicated that some extracts prepared from medicinal plants exhibit high anti-elastase activities, these included white tea which was found to have very high phenolic content (0.77 mg GAE/ml). This extract caused 89% inhibition which recorded the highest as compared to other tested plants (Thring et al., 2009). Triterpenoids known as boswellic acids isolated from frankincense (*Boswellia* spp.) (Family: Burseraceae) resin showed anti-elastase activity (Melzig et al., 2001). Rosemary extracts from *R. officinalis* L. (Family: Lamiaceae) have also been reported to have high anti-elastase activity (Baylac and Racine, 1999). A quinazolinone alkaloid isolated from the fruits of *Evodia officinalis* (Dode) Huang (Family: Rutaceae) have been reported to have MMP-1 inhibitory activity (Jin et al., 2008). Recently, *Cucumis sativus* L. (Family: Cucurbitaceae) fruit has been found to possess *in vitro* inhibition of elastase and MMP-1, which suggested the potential of this plant as anti-wrinkle (Nema et al., 2011). Studies carried out by Chen et al. (2010) have demonstrated that the compound was 6  $\alpha$ -hydroxycurcumanolide (5; Figure 2). New sesquiterpene isolated from the rhizome of *Curcuma longa*, inhibited fMLP/CB-induced elastase release with  $IC_{50}$  values  $\leq 6 \mu\text{M}$  (Chen et al., 2010). Sesquiterpenes with monoterpenes are an important constituent of essential oils in plants. They are the most diverse group of isoprenoids. Sesquiterpenes structures present several acyclic, mono-, bi-, tri-, and tetracyclic systems. Avarol and its quinone derivative avarone (6; Figure 2) were isolated from a

Mediterranean (*Dysidia avara*) and an Australian sponge (*Dysidia* species) (Nema et al., 2011). These products have been reported as potent and selective inhibitors of HLE and the potency is dependent on two important factors, namely, the R4 substituent and the nature of the leaving group positioned on the nitrogen attached methylene of benzisothiazolone nucleus (7; Figure 2).

Studies conducted by Parimala et al. (2011), indicated that thymol (Thy) (figure 2.8) did not show any cytotoxic effect in normal human peripheral blood mono nuclear cell. After the incubation of neutrophils with increasing amounts of Thy (2.5, 5, 10, 20  $\mu\text{g/ml}$ ), the results showed that Thy inhibited fMLP-induced elastase release in a concentration-dependent manner, with the effects of 10 and 20  $\mu\text{g/ml}$  being statistically significant. Several reports have demonstrated that the main components of essential oil extracted from *N. sativa* (L.) seeds showing inhibitory effects on HNE activity are monoterpenes including p-cymene (37.3%), TQ (13.7%), carvone (0.9%), Thy (0.33 %) and carvacrol (11.77 %). These compounds comprise 65% of total essential oil; it has been reported that the active constituents isolated from *N. sativa* essential oil inhibited HNE with different  $IC_{50}$  values. TQ (9; Figure 2), inhibits HNE activity with an  $IC_{50}$  value of 30  $\mu\text{M}$ . The  $IC_{50}$  value of carvacrol was the lowest (12  $\mu\text{M}$ ). From these results, it was clear that the test compounds inhibited HNE activity with different  $IC_{50}$  values, but carvacrol inhibited HNE with a very low  $IC_{50}$  value (12  $\mu\text{M}$ ). The inhibitory concentration of Thy was the highest at 104  $\mu\text{M}$ . Although some studies indicated that TQ is the most active molecule that could be responsible for the effects of essential oil, the results of our studies, clearly indicated that this compound inhibited HNE activity with an  $IC_{50}$  value (12  $\mu\text{M}$ ) about 3-fold that of carvacrol. It is clear that carvacrol, which has a hydroxyl group in position 3 on the benzene ring, was the most bioactive molecule in inhibiting HNE activity. Because a specific substrate was used in this study, the inactivation of HNE could be explained by the competition of this compound and substrate for the same specific binding sites. About 2-fold progressive inhibition of HNE was observed by carvacrol compared with Thy. Inhibition of HNE activity by carvacrol was explained by its direct binding with the enzyme, forming an enzyme-inhibitor complex. Taking into consideration the  $IC_{50}$  values of each compound, carvacrol is a potent inhibitor of HNE (Kacem and Meraihi, 2006). Studies conducted by Umar and his co-authors (2012), reported that TQ was effective in bringing significant changes on all the parameters studied including articular elastase; the report concluded that the administration of TQ may have potential value in the treatment of inflammatory disease (Umar et al., 2012). The effects of three prenylhydroquinone glucosides and four caffeoylquinic esters obtained from *Phagnalon rupestre*, on elastase release from PMNL was tested by Luis (2002). 4, 5-Dicaffeoylquinic acid strongly inhibited elastase release with an  $IC_{50}$  value of 4.8  $\mu\text{M}$ . A

conclusion was raised that caffeoyl conjugates with either quinic acid or a hydroquinone glycoside, exert notable activity on some of the biological functions of PMNL implicated in the initiation and maintenance of inflammation, mainly liberation of hydrolytic enzymes including elastase. Recent studies carried out by Alasbahi and Melzig (2008) using 15 extracts of different polarities were tested for their inhibitory effects against the activity of HNE (EC 3.4.21.37). Among the plants tested, *A. helianthoides* was the most active inhibitor of HNE (Alasbahi and Melzig, 2008). The dichloromethane extract of this medicinal plant showed the most active inhibitory effect on the HNE activity ( $IC_{50} = 0.4 \mu\text{g/ml}$ ). The observed HNE inhibitory effect of the tested extracts might be partially attributed to the phenolic compounds. The MeOH extract of *Ilex paraguariensis* leaves showed strong HNE inhibitory effect. Bioassay-guided fractionation led to the isolation of a new pyrrole alkaloid, along with seventeen known compounds from the MeOH extract of *I. paraguariensis* leaves, and their chemical structures were elucidated on the basis of spectroscopic analysis. All isolated compounds were evaluated for HNE inhibitory activity, and the result demonstrated that dicaffeoylquinic acid derivatives and flavonoids exhibited potent HNE inhibitory activity with  $IC_{50}$  values ranging from 1.4 to 7.3  $\mu\text{M}$  (Guang-Hua et al., 2009). As a conclusion from these studies, the extracts from the described medicinal plants found to have very high phenolic content and their purified constituents mainly the phenols have been shown to have beneficial therapeutic potential for many diseases, such as COPD and emphysema. Many research reports have indicated that phenolic compounds extracted from medicinal plants inhibits the activity of HNE. The observed inhibition was due to the presence of phenolic compounds mainly monoterpenes which inhibit HNE in a dose dependent manner. Some phenolic compounds such as carvacrol, benzisothiazolone, 5-isopropyl-2-methyl phenol, dicaffeoylquinic acid derivatives and flavonoids are potent inhibitor of HNE, and could be considered as natural anti-elastase agents in the treatment of injuries that appear in chronic obstructive pulmonary disease and emphysema. However, further studies may be required to confirm the reported results especially in the case of emphysema conditions.

## ABBREVIATIONS

**ARDS**, Acute respiratory distress syndrome;  **$\alpha$ 1-AT**,  $\alpha$ 1-antitrypsin; **CE**, capillary electrophoresis; **CF**, cystic fibrosis; **CMT**, chemically modified tetracycline; **COPD**, chronic obstructive pulmonary disease; **fMLP**, N-formyl-methionine-leucine-phenylalanine; **GAE**, gallic acid equivalents; **HLE**, human leukocyte elastase; **HNE**, human neutrophil elastase;  **$IC_{50}$** , inhibitory concentration; **MAE**, microwave assisted extraction; **MMPs**, matrix metalloproteinases; ***N. sativa***, *Nigella sativa*; **NSPs**,

neutrophil serine proteases; **PAF**, platelet activating factor;  **$\alpha$ 1-PI**,  $\alpha$ 1-protease inhibitors; **PLE**, pressurized liquid extraction; **PMNL**, polymorphonuclear leukocyte; **ROS**, reactive oxygen species; **SFE**, supercritical fluid extraction; **SLPI**, secretory leukoproteinase inhibitor; **SOD**, superoxide dismutase; **SSF**, solid state fermentation; **SPis**, serine protease inhibitors; **TEAC**, trolox equivalent anti-oxidant capacity; **Thy**, thymol; **TQ**, thymoquinone; **VC**, vital capacity.

## REFERENCES

- Alasbahi R, Melzig M (2008). The *In Vitro* Inhibition of Human Neutrophil Elastase Activity by some Yemeni Medicinal Plants. *Sci. Pharm.* 76:471-483.
- An B, Kwak J, Park J, Lee J, Park T, Lee J, Son J, Jo C, Byun M (2005). Inhibition of enzyme activities and the antiwrinkle effect of polyphenol isolated from the Persimmon Leaf (*Diospyros kaki folium*) on human skin. *Dermatol. Surg.* 31:848-855.
- Araújo CL, Bezerra IWL, Oliveira AS, Macedo LLP (2005). In vivo bio insecticidal activity to *Ceratitidis capitata* (fruit fly) and *Callosobruchus maculatus* (cowpea weevil) and in vitro bioinsecticidal activity towards different orders of insect pests of a trypsin inhibitor purified from Tamarind tree (*Tamarindus indica*) seeds. *J. Agric. Food.Chem.* 53:4381-4387.
- Barnes PJ, Shapiro SD, Pauwels RA (2003). Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur. Respir. J.* 22:672-688.
- Baylac S, Racine P (1999). Inhibition of human leukocyte elastase by natural fragrant extracts of aromatic plants. *Int. J. Aromather.* 14:179-182.
- Benthin B, Danz H, Hamburger M (1999). *J. Chromatogr.* A837:21169.
- Chen JJ, Jih-Jung C, Cheng-Shuan T, Hwang T, Shieh P, Sung P (2010). Sesquiterpenes from the rhizome of *Curcuma longa* with inhibitory activity on superoxide generation and elastase release by neutrophils. *Food. Chem.* 119:974-980.
- Dey S, Deepak M, Setty M, D'Souza P, Agarwal A, Sangli GK (2009). Bioactive caffeic acid esters from *Glycyrrhiza glabra*. *Nat. Prod. Res.* 23:1657-63.
- Grabley S, Thiericke R (1999). Bioactive agents from natural sources: trends in discovery and application. *Adv. Biochem. Eng. Biotechnol.* 64:101-54.
- Greer RM, Butain HM, Lewindon PJ, Wainwright CE (2004). Vitamin A levels in patients with CF are influenced by the inflammatory response. *SC. J. Cyst. Fibros.* 3:143-149.
- Gu Y, Lee HM, Simon SR, Golub LM (2011). Chemically modified tetracycline-3 (CMT-3): a novel inhibitor of the serine proteinase, elastase. *Pharmacol. Res.* 64(6):595-601.
- Guang-Hua X, Young-Hee K, Soo-Jin C, In-Ja R (2009). Chemical constituents from the leaves of *Ilex paraguariensis* inhibit human neutrophil elastase. *Arch. Pharm. Res.* 32:1215-220.
- Hayakawa M, Katabami K, Wada T, Sugano M, Hoshino H, Sawamura A, Gando S (2010). Sivelestat (selective neutrophil elastase inhibitor) improves the mortality rate of sepsis associated with both acute respiratory distress syndrome and disseminated intravascular coagulation patients. *Shock* 33(1):14-8.
- Herrero M, Mendiola JA (2010). Supercritical fluid extraction: recent advances and applications. *J. Chromatogr.* 217:2495-2511.
- Hiemstra PS, Eisenhauer PB, Harwig SS, Barselaar MT (2002). Antimicrobial proteins of murine macrophages. *Infect. Immun.* 61(7):3038-3046.
- Jin HZ, Du JL, Zhang WD, Yan SK, Chen HS, Lee JH, Lee JJ (2008). A new quinazolinone alkaloid from the fruits of *Evodia officinalis*. *Fitoterapia* 79:317.
- Kacem R, Meraihi Z (2006). Effect of essential oil extracted from *Nigella sativa* (L.) seeds and its main components on human neutrophil elastase activity. *J. Pharm. Soc. Japan* 126(4):301-305.
- Kacem R (2011). Effects of *Nigella* (*Nigella sativa* L.) seed extract on human neutrophil elastase activity. Editors: Preedy VR, Watson RR



- Patel VB, Nuts and Seeds in Health and Disease Prevention (1st Ed.) (pp 823- 829). London, Burlington, San Diego: Academic Press.
- Kawabata K, Hagio T, Matsuoka S (2002). The role of neutrophil elastase in acute lung injury. *Eur. J. Pharmacol.* 451:1-10.
- Kawamura F, Kikuchi Y, Ohira T, Yatagai M (1999). Accelerated solvent extraction of paclitaxel and related compounds from the bark of *taxus cuspidata*. *J. Nat. Prod.* 62:244-271.
- Khan MS, Ahmad I (2011). *in vitro* antifungal, anti-elastase and anti-keratinase activity of essential oils of *Cinnamomum*, *Syzygium* and *Cymbopogon* species against *Aspergillus fumigatus* and *Trichophyton rubrum*. *Phytomedicine* 19(1):48-55.
- Kim Y, Uyama H, Kobayashi S (2004). Inhibition effects of (+)-catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. *Biochem. Biophys. Res. Commun.* 320:256-261.
- Korkmaz B, Moreau T, Gauthier F (2008). Neutrophil elastase, proteinase 3 and cathepsin G: physicochemical properties, activity and physiopathological functions. *Biochimie* 90:227-242.
- Kori S, Namiki H, Suzuk K (2009). Biphasic regulation of polymorphonuclear leukocyte spreading by polyphenolic compounds with pyrogallol moieties. *Int. Immunopharmacol.* 9:1159-1167.
- Luis G (2002). Effects of caffeoyl conjugates of isoprenyl-hydroquinone glucoside and quinic acid on leukocyte function. *Life Sci.* 71:2995-3004.
- Martins S, Solange A, Mussatto I (2011). Teixeira a Bioactive phenolic compounds: Production and extraction by solid-state fermentation. *Biotechnol. Advan.* 29:365-373.
- Melzig MF, Löser B, Ciesielski S (2001). Inhibition of neutrophil elastase activity by phenolic compounds from plants. *Pharmazie* 56:967-970.
- Newman DJ, Cragg GM (2007). Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* 70:461-477.
- Nema NK, Maity N, Sarkar B, Mukherjee PK (2011). *Cucumis sativus* fruit potential antioxidant, anti-hyaluronidase, and anti-elastase agent. *Arch. Dermatol. Res.* 303: 247-252.
- Nicola L, Mather A, Anand D, Padmakumar A, Richard MB (2012). Emphysema, lung volume reduction and anesthesia. *Trends in Anaesthesia and Critical Care.* In press. doi:10.1016/j.tacc.2012.03.005.
- Owen CA, Campbell MA, Sannes PL, Boukedes SS, Campbell EJ (1995). Cell surface-bound elastase and cathepsin G on human neutrophils. A novel, nanoxidative mechanism by which neutrophils focus and preserve catalytic activity of serine proteinases. *J. Cell Biol.* 131:775-789.
- Parimala G, Saravana S, Chakraborty T (2011). Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. *Chemico-Biological Interactions.* 193:97-106.
- Raja TA (2006). Alpha<sub>1</sub>-Antitrypsin Deficiency. *Respir. Med.* 1:80-87.
- Ram R, Balachandar S, Vijayananth P, Singh VP (2011). Medicinal plants useful for treating chronic obstructive pulmonary disease (COPD): Current status and future perspectives. *Fitoterapia* 82:141-151.
- Robert AC (2005). *A Guide for Medicinal Chemists and Pharmacologists.* Wiley, Germany.
- Snider GL, Lucey EC, Stone PJ (1994). Pitfalls in Antiprotease therapy of emphysema. *Am. J. Respir. Crit. Care Med.* 150:S131-S137.
- Stockley RA (1999). Neutrophils and protease antiprotease imbalance. *Am. J. Respir. Crit. Care Med.* 160:S49-S52.
- Stone PJ, Lucey EC, Snider GL (1990). Induction and exacerbation of emphysema in hamsters with human neutrophil elastase inactivated reversibly by a peptide boronic acid. *Am. Rev. Respir. Dis.* 141:47-52.
- Thring TSA, Pauline Hill, Declan P (2009). Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complement Altern. Med.* 2009:9:27.
- Umar S, Zargan J, Umar K (2012). Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. *Chem-Biol. Inter.* 197:40-46.
- Vasconcelos A, Azoia NG, Carvalho AC, Gomes AC, Güebitz G (2011). Tailoring elastase inhibition with synthetic peptides. *Eur. J. Pharmacol.* 666:53-60.
- Vatai TM (2009). Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. *J. Food Eng.* 90:246-254.
- WHO (2008). "Fact sheet no. 134: Traditional medicine". 2008-12-01. Retrieved 200.
- Willemse BW, Postma DS, Timens W, Ten Hacken NH (2004). The impact of smoking cessation on respiratory symptoms, lung function, airway hyper-responsiveness and inflammation. *Eur. Respir. J.* 23:464-476.