Review of Angiotensin-converting Enzyme Inhibitory Assay: Rapid Method in Drug Discovery of Herbal Plants

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ABSTRACT
The renin-angiotensin-aldosterone system is a signaling pathway which responsible in the blood pressure regulation. Angiotensin-converting enzyme (ACE) is one of the key elements responsible for the hypertensive mechanism. It converts angiotensin-I to angiotensin-II. The discovery history of the ACE inhibitory activity assay method has been through a long stage for decades and development continues until today. The ACE inhibitory activity has become an effective screening method in the search for new antihypertensive agents from herbal plants. Some of in vitro assay methods were used to examine the activity of ACE inhibitors based on the substrate usage, such as; Cushman and Cheung Method using a substrate hippuryl-histidyl-leucine (HHL), Holmquist method using a substrate furanacryloyl-tripeptide, Ebl and Wagner method using a substrate benzoyl-[l-14C] glicyl-1-histidine-1-leucine, Carmel and Yaron method using a substrate o-aminobenzoicyl-glycyl-p-nitrophenylalanilproline, and Lamb method using 3-hydroxybutyryl-glycyl-glycyl-glycine as substrate. Several different methods to measure the results of enzymatic reactions or separating substrate with products, including spectrophotometric, fluorometric, high-performance liquid chromatography, electrophoresis, and radiochemistry. Application of the test method for screening the ACE inhibitors activity and investigation of active compounds from natural products can be done easily with this method, it is very helpful in research because the results obtained are simple, accurate, and rapid.

Key words: Angiotensin-converting enzyme inhibitory activity, angiotensin-converting enzyme, herbal plants, in vitro assay method, Renin-angiotensin-aldosterone system

INTRODUCTION
Initially, the screening for antihypertensive effect in the drugs discovery from natural products mainly used empirically been done over the years and have used several experiments on animal models. Studies in the drug discovery, especially as an antihypertensive has developed rapidly since the discovery of the angiotensin-converting enzyme (ACE). The ACE converts angiotensin decapeptide inactive into active octapeptide angiotensin II in the kidneys, especially in the renin-angiotensin-aldosterone system. The activity of ACE inhibitory by in vitro has become an effective assay method in the drugs discovery as antihypertensive. This has been demonstrated by comparing the assay method of seven kinds medicines (captopril, enalapril, zofenopril, ramipril, fosinopril, lisinopril, and SQ 29852) as the ACE inhibitor. However, in studies, the activity of ACE inhibitory for a positive control is more widely used a captopril because the drug is most widely used as antihypertensive and heart failure, and also have a free radical scavenger activity are highly relevant as an ACE inhibitor.

In modern medicine, the drug discovery has become more specific and focus on particular target objectives. The identification of receptor or enzyme as a molecular target which has an important role in the disease regulation and then performs searches the ligand or substrate or inhibitor of a specific target is the reason behind this approach. The discovery of a new drugs mainly from natural materials are directly aimed at the molecular target (receptor or enzyme) is more effective and efficient than conventional methods using animal model experiments performed with the treatment and observation in general, and require treatment and observation are more complicated if performed on specific targets (e.g. receptors or enzymes), as well as the type of the test sample to be used. Considering the potential of natural resources are abundant so that the necessary a special strategy conduct research one of which is an assay method of the ACE inhibitors activity in vitro. The present studies review aims to highlight the discover history, assay methods, and application in natural products drugs discovery of ACE inhibitory activity assay.

MATERIALS AND METHODS
This paper reviews about the ACE Inhibitory Activity Assay from comprehensive literature. The literature was searched between August and October 2016 from the electronic databases including PubMed, Scopus, ScienceDirect, and Google Scholar.

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RESULTS AND DISCUSSION

The discover history of in vitro angiotensin-converting enzyme inhibitory assay methods

The assay method of ACE activity in vitro was begun in 1954–1957, when Skeggs et al. succeeded in isolating and purifying the enzyme which can hydrolyze decapetide angiotensin I, then release vasopressive octapeptide angiotensin II and histidine-leucine dipeptide inactive or commonly referred to as “converting enzyme” from the horses plasma. [14]

From the results of the discovery, the action mechanism of this enzyme can be determined [Figure 1]. [15] However, at that time, there has been no progress on the development of in vitro assay methods. About eleven years later (1968–1969) with the discovery of radiometric assay using the labeled angiotensin I substrate, wherein the release of radioactive histidine-leucine which serves as an enzymatic activity index [16] and further developed methods for chemical assay of the ACE, where the enzymatic reaction product based on the determination of histidine-leucine with fluorometric method on different substrates. [17] In 1970–1971, Cushman and Cheung managed to find a spectrophotometric assay method for measuring the amount of ACE to produce hippuric acid (HA) from hippuryl-histidyl-leucine (HHL) as substrate. [18]

Carmel and Yaron (1977–1978) developed a measurement method of the ACE inhibitory activity using an O-aminobenzoylglycine-p-nitrophenylalanylproline as a substrate and then hydrolyzed into O-aminobenzoylglicyl. [19] At the same time, Hayakari et al., developed assay methods of the ACE inhibitory activity in a spectrophotometry manner using HHL as substrate and a colorimetric reagent of HA namely 2,4,6-trichloro-s-triazine (TT). [20]

In 1979–1991, some research reported the usage of a substrate other than HHL for assay method of ACE inhibitory activity such as Holmqquist et al. using tripeptide furanacryloyl (FA-PGG) as a substrate, [21] Baudin et al., using benzoyl-[l-14C] glycyl-L-histidyl-L-leucine as a substrate, [22] and the usage a substrate of chromophore- and fluorophore-labelled tripeptide danyltriglycine by Elbl and Wagner. Since 1993, Doig and Smiley have developed method of ACE inhibitory activity assay using a shielded hydrophobic phase (SHP) column for high-performance liquid chromatography (HPLC) instrument and HHL substrate. [24] Nakamura et al. have performed purification and characterization of ACE inhibitory using HPLC instrument. [25]

Initially, all methods are constantly being developed is only used against the pure compound and has not been in use on samples containing multi-compound as in plant extracts. However, Hansen et al. began to apply this method on the plant extracts. [26] In 2007, Lam et al., managed to synthesize and use a substrate 3-hydroxybutyrylglycyl-glycyl-glycine (3HB-GGG) in the assay method of the ACE inhibitory activity, [27] and this method is also applicable to samples of plant extracts.

In vitro angiotensin converting enzyme assay methods

Several assay method of ACE inhibitory activity can be used to detect the activity of ACE inhibition from drugs or plant extract. Each method is distinguished by the use of substrates and measurement methods of enzymatic reaction products or separation of the substrate with the products. In addition, the author provides the names of each method based on the inventor names, ACE inhibitory activity test method is divided into several methods as follows:

Cushman and Cheung method

Cushman and Cheung (1970–1971) have developed the assay method of the activity of ACE inhibitors using a substrate hippuryl-histidyl-leucine (HHL), the ACE will hydrolyze HHL into HA [Figure 2]. [28] The HA was measured at a wavelength of 228 nm to describe the ACE activity using a ultraviolet-visible (UV-Vis) spectrophotometer instrument. When there is an ACE inhibitor, the concentration of HA formed will be reduced. [18]

The success of Cushman and Cheung’s method of assay the ACE inhibitors activity still depends on the ability to separate HA formed from the HHL substrate. Therefore, the another approach is required to facilitate the use of these methods optimally, among other: (a) The addition of TT in the sample mixture containing HA, a reaction between TT with HA concentration of HA formed will be reduced. [29] (b) The usage of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) to stop the reaction and Cyanuric chloride in 1,4-dioxane as a color reagent measured in photometry at a wavelength of 504 nm. [29] (c) The addition of D-Phthalaldehyde which then react with the substrate hydrolysis histidine-leucine is measured by a fluor-o-colorimeter instrument at a wavelength of 495 nm emission and 365 nm excitation. [30] (d) The use of benzene-sulfonyl-chloride (BSC) as a color reagent in the presence of...
Dansyltriglycine hydrolysis by angiotensin-converting enzyme

The principle of the assay method of angiotensin-converting enzyme inhibitors activity using angiotensin-converting enzyme kit-Water-Soluble Tetrazolium Salt 1

Baudin method

Baudin et al. have developed a method with radiometric technology to measure ACE activity in urine and using a substrate that has been labeled namely benzyol-[l-14C] glycyL-L-histidyl-L-leucine. The substrate will be cleaved at the glycine-histidine bonding and form benzyol-[l-14C] glycine. This test method can be used in the study of ACE in the urine as a marker if there is kidney damage.[22]

Carmel and Yaron method

The Carmel and Yaron methods began to be discovered and developed in 1978, an assay method of the ACE inhibitory activity using o-amino benzyol glycyL-p-nitrophenalanilproline as a substrate then hydrolyzed into o-amino benzyol glycyL as a compound fluorescence, for discontinue enzymatic reaction can be used EDTA.[19] In 2006, Sentandreu and Toldrà using this method with slight modifications were measured using a multi-scanning microplate fluorometer. The advantage of this method is the usage capacity of large sample and in a short time.[48] However, this method is not widely used.

Lam method

Lam le et al., begun to study in 2007 managed to find a new substrate is 3HB-GGG for assay method of the activity of ACE. The 3HB-GGG is cleaved into Gly-Gly-Gly amino acid and 3-hydroxybutyric acid (3HB) by the ACE. Then 3HB measured using F-kit. This method is more sensitive, rapid, accurate, and suitable for conventional methods.[27] Then, Lam le et al. carried out development using a Water-Soluble Tetrazolium Salt (WST1) to detect 3-hydroxybutyrate formed.[46] The mechanism of the assay method of the ACE inhibitor activity using ACE kit-WST1 described in Figure 4 and this method using flow injection analysis to detect directly in a rapid, simple, and accurate.[47] This enzyme has been created in the form of a kit which has been patented with the name the ACE kit-WST1. The LAM method with the 3HB-GGG substrate using the ACE kit-WST1 can also be applied using microplate ELISA reader.[48-50]

Applied of the angiotensin converting enzyme inhibitory assay method in drug discovery of natural products

The screening of antihypertensive activity on natural products, especially herbs used empirically as antihypertensive has been conducted over the years. The application of the assay method of the activity of ACE inhibitory for the activity screening in the natural product research is very helpful because the results obtained in a rapid, accurate, and simple. In general, the methods described above have been conducted optimization for the type and the materials concentration used so that the reaction mechanism of the enzyme occurs the same as in the actual state in the body.[29,30] this method also was performed standarization[30]
Table 1: The results data of angiotensin-converting enzyme inhibitors activity in vitro from plants

| Species of plant | Family | Part of plant | ACE-I activity (%) | References |
|------------------|--------|---------------|--------------------|------------|
| **Species from India** | | | | |
| Abutilon indicum G. Don | Malvaceae | Root | 18 | [26] |
| Achyranthes aspera L. | Amaranthaceae | Arial parts | 19 | |
| Boerhavia diffusa L. | Nyctaginaceae | Root | 40 | |
| Cardiospermum halicacabum L. | Sapindaceae | Arial parts | 8 | |
| Centella asiatica L. | Apiaceae | Arial parts | 73 | |
| Desmodium gangeticum DC. | Fabaceae | Root | 27 | |
| Entada pursaetha DC. | Fabaceae | Seed | 34 | |
| Merremia tridentata Dennst. | Convolvulaceae | Arial parts | 30 | |
| *Pseudarthria viscosa* W and Arn. | Fabaceae | Root | 71 | |
| *Sida* acuta | Malvaceae | Root | 32 | |
| *Sida* cordifolia L. | Malvaceae | Root | 38 | |
| *Sida* retusa L. | Malvaceae | Root | 41 | |
| *Triumfetta rhomboidea* Jacq. | Tiliaceae | Root | 61 | |
| **Species from South Africa** | | | | |
| *Pseudarthria hookeri* W and Am. | Fabaceae | Root | 90 | |
| **Species from China** | | | | |
| Aristolochia manshuriensis Kom | Aristolochiaceae | Seed | 36 | |
| Crataegus pinnatifida Bunge | Rosaceae | Fruit | 7 | |
| Desmodium styracin Mer. | Fabaceae | Leaf, seed | 39 | |
| *Houttuynia cordata* Thunb. | Saururaceae | Whole plant | 23 | |
| *Plantago asiatica* L. | Plantaginaceae | Whole plant | 35 | |
| *Pueraria lobata* Ohwi | Fabaceae | Root | 38 | |
| *Pyrrosia lingua* Farwell | Polypodiaceae | Whole plant | 24 | |
| **Species from Chile** | | | | |
| *Aristolitia chilensis* DC. | Elaeocarpaceae | Bark | 36 | |
| *Escallonia myrtoides* (L.) Hook. | Grossulariaceae | Arial part | 15 | |
| *Fuchsia magellanica* L. | Onagraceae | Arial part | 47 | |
| *Geranium core‑core* L. | Geranaceae | Arial part | 33 | |
| *Gunnera tinctoria* L. | Gunneraceae | Leaf | 57 | |
| *Hexachlamys edulis* Berg. | Myrtaceae | Leaf | 91 | |
| *Quichamalium chinensis* Molina | Santalaceae | Arial part | 43 | |
| *Schinus latifolius* L. Schinus | Anacardiaceae | Bark | 74 | |
| *Adenopodia spicata* (E. Mey.) Pres | Fabaceae | Leaf, root | 97,8 | [41] |
| *Adiantum capillus‑veneris* L. | Pteridaceae | Leaf | 13 | |
| *Agapanthus africanus* L. | Alliaceae | Leaf, root | 63 | |
| *Agave americana* L. | Asparagaceae | Leaf | 82 | |
| *Catha edulis* (Vahl) Forssk. | Celastraceae | Leaf | 82 | |
| *Cannabis sativa* L. | Cannabaceae | Leaf, root | 18 | |
| *Clausena anisata* (Wild.) Hook. | Rutaceae | Leaf | 54 | |
| *Dietes iridioides* (L.) Sweet | Iridaceae | Leaf, root | 80 | |
| *Drimia robusta* Bak. | Asparagaceae | Leaf, bulbs | 23 | |
| *Eucalyptus robusta* (Hochst.) | Pentapetalaceae | Leaf, bark | 83 | |
| *Ekebergia capensis* Sparrow | Meliaceae | Leaf | 37 | |
| *Hypoxyis cicliophyla* Bak. | Hypoxidaceae | Leaf | 37 | |
| *Mesembryanthemum sp.* | Aizoaceae | Leaf, stem | 90 | |
| *Prototus longifolia* (Bernh. | Anacardiaceae | Leaf | 77 | |
| *Rhus shirinensis* Bak. F. | Anacardiaceae | Leaf, bark | 85 | |
| *Sclerocephra birrea* (A. Rich.) | Anacardiaceae | Leaf | 68 | |
| *Stangeria eriopus* (Kunze) Baill. | Stangeriaceae | Leaf | 55 | |
| *Tulbaghia violacea* Harv. | Amaryllidaceae | Leaf, root | 72 | |
| *Turraea floribunda* Hochst. | Meliaceae | Leaf | 45 | |
| *Cecropia guianensis* | Coccolobaucaceae | Stem | 20 | [42] |
| *Persea americana* Miller | Lauraceae | Stem | 31 | |
| *Croton antisyphiliticus* Mart. | Euphorbiaceae | Leaf | 32 | |
| *Combretum fruticosum* Stuntz | Combretaceae | Leaf | 38 | |
| *Buddleja stachyoides* | Loganiaceae | Leaf | 31 | |
| *Ouratea semiserrata.* | Ochnaceae | Stem | 68 | |
| *Tripogandra elata* D. R. Hunt | Commelinaceae | Leaf | 32 | |
| *Commelina diffusa* Burm. F | Commelinaceae | Leaf | 45 | |
| *Tradescantia zebrina* Hort. | Commelinaceae | Leaf | 35 | |
| *Hymenaea courbaril* L. | Leguminosae | Resin | 10 | |
| *Maytenus ilicifolia* Mart. | Celastraceae | Leaf | 19 | |

Contd...
and validation,\textsuperscript{[48]} between each method as well as with conventional methods performed in vivo method. Selection of assay methods based on the substrate type depending on the availability of instruments that can be used to measure the inhibitory activity of ACE from the samples.\textsuperscript{[51]} Some herbs that have been carried out the screening of activity using the \textit{in vitro} assay method of ACE inhibitors activity as shown in Table 1. Based on the screening results of ACE inhibitors activity from various species of plant, the empirical usage of natural products such as the celery plant (\textit{Apium graveolens}) as antihypertensive and isolation of the junipediol A is responsible as ACE inhibitors. The search of active compound can be performed easily with this method, in general, can be started from screening the activity of the extract, fractions, and isolates. Even for the isolates that have been characterized and determined the structure can be more optimal if combined with the \textit{in silico} assay method\textsuperscript{[13,64]} to obtain a prediction overview of the action mechanism and the group most responsible of the compounds studied. Several studies based on the empirical usage of natural products such as the celery plant \textit{(Apium graveolens)} as antihypertensive and isolation of the junipediol A 8-\textit{OβD}-glucoside (1-\textit{βD}-glucosylxy-2- (3-methoxy-4-hydroxyphenyl)-propane -1,3-diol) compound has the strongest activity as an ACE inhibitor compared to another known compound (such as, 11,21-\textit{dioxo-3

| Species of plant | Family | Part of plant | ACE-I activity (µM) | References |
|-----------------|--------|---------------|---------------------|-----------|
| \textit{Acalypha hispida} | \textit{Acanthaceae} | Leaf | 100 | [43] |
| \textit{Annona squamosa} | \textit{Annonaceae} | Seed | 100 | [40] |
| \textit{Asparagus officinalis} | \textit{Asparagaceae} | Leaf | 0.40 | [52] |
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 40 | [56] |
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 40-60 | |
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 60-100 | |
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [6] |
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [
| \textit{Asperula odorata} | \textit{Asperulaceae} | Leaf | 16 | [

\textbf{Table 1: Contd...}

| Species of plant | Family | Part of plant | ACE-I activity (µg/ml) | References |
|-----------------|--------|---------------|---------------------|-----------|
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |
| \textit{Betula alba} | \textit{Betulaceae} | Leaf | 500 | [57] |

\textbf{ACE-I: Angiotensin-converting enzyme inhibitory

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CONCLUSION

By the discovery of the various assay methods of ACE inhibitors activity, this method is very effective for testing on natural products so that the researchers can perform quickly, accurately, and simply, so they can more focus on the development of extraction, fractionation, and isolation methods for the enrichment and dereplication of the active compounds. The selection of the assay methods for determining the inhibitory activity of ACE is only based on subject to availability of the substrate and the measurement instruments for example spectrophotometric, photo fluorometer, HPLC, and others.

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Conflicts of interest

There are no conflicts of interest.

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