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REVIEW ARTICLE

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, Elbl and Wagner method using a substrate benzoil-[l-14C] glicyl-L-histidine-L-leucine, Carmel and Yaron method using a substrate o-aminobenzoylglycyl-p-nitrophenylalanilproline, and Lam method using 3-hydroxybutyrylglycyl-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.[1-3] 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.[4-8] 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.[9] 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.[10]

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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.[2,9,11-13] 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 decapeptide 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 Holmquist *et al.* using tripeptide furanacryloyl (FA-PGG) as a substrate,[21] Baudin *et al.*, using benzoyl-[l-14C] glicyl-L-histidyl-L-leucine as a substrate,[22] and the usage a substrate of chromophore- and fluorophore-labelled tripeptide dansyltriglycine[23] 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 le *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.

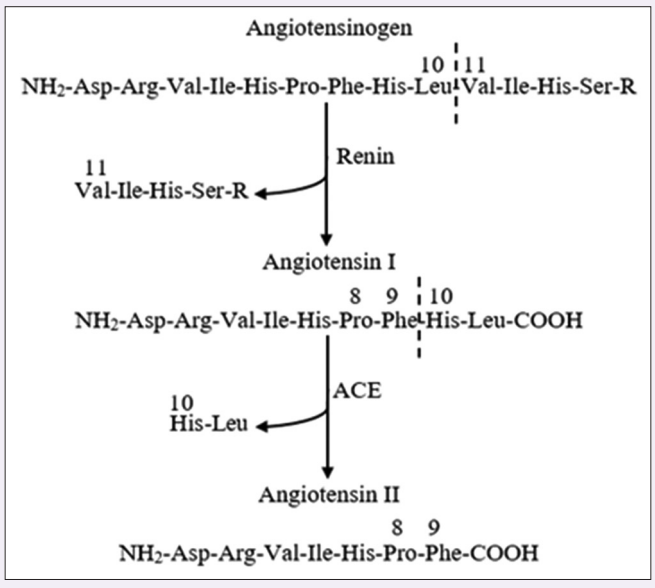

Figure 1: Scheme of angiotensin II formation by angiotensin-converting enzyme

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 formed should be measured at a wavelength of 382 nm.[20] (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 o-Phthaldialdehyde which then react with the substrate hydrolysis histidine-leucine is measured by a fluoro-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

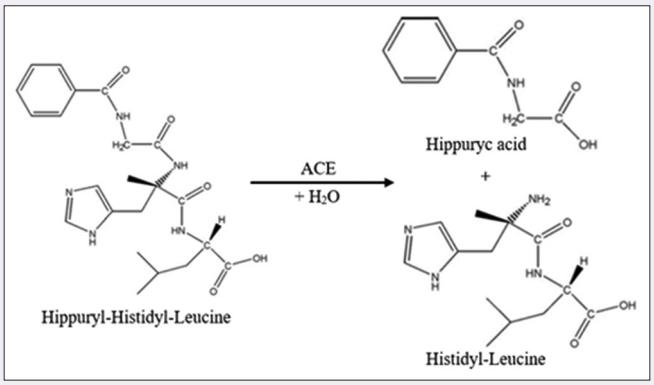

Figure 2: HHL hydrolysis by angiotensin-converting enzyme

quinoline[31] (referred to as BSC-visible spectrophotometry method) and then modified using a microtiter plate reader.[32]

Besides using the UV-Vis spectrophotometer, can also use the high HPLC, however, require some modifications such as the use of SHP HPLC to separate HHL and HA, which can be injected directly for the measurement of the HA absorbance using a spectrophotometer wavelength at 254 nm with a 8.0 mL flow-cell.[24] Furthermore, Mehanna and Dowling, were able to develop a more simple HPLC method,[33] which further developed by using reverse-phase-HPLC (RP-HPLC) and HEPES as a buffer.[34]

Holmquist method

This method was first introduced by Holmquist *et al.* in 1979 using FA-PGG as a substrate. This method is based on the absorption spectrum blue shift that occurred on the substrate hydrolysis produces dipeptide and furanacryloyl-blocked amino acid.[21] This was confirmed by Lundberg *et al.*, to perform measurements of the ACE inhibitory activity in serum using an FA-PGG substrate.[35] This method has been conducted optimization, validation, and modifications of the FA-PGG usage as a substrate for screening bioactive peptides.[36,37] The FA-PGG substrate is hydrolyzed into a dipeptide (glycylglycine) and furanacroyl-phenylalanine by ACE and measured at a wavelength of 328 nm and 352 nm,[37,38] the addition of ethylenediaminetetraacetic acid (EDTA) aimed to stopping the enzymatic reaction[37] and other modifications using a microplate reader.[38] Lahogue *et al.* has reported the application of HPLC-UV method for the determination of the activity of ACE inhibitors.[39]

Elbl and Wagner method

This method was developed by Elbl and Wagner in 1991 with the usage of the chromophore- and fluorophore-labeled tripeptide dansyl triglycine as substrate, which is cleaved by the ACE into dansylglycine and diglycine [Figure 3].[23] Furthermore, this method modified by using a microtitre plate which is injected directly into the UV detection.[26,40] Duncan *et al.* have been applying this method for screening the activity of ACE inhibitors from Zulu medicinal plants.[41]

Lacaille-Dubois *et al.* modify Elbl and Wagner method in which the hippuric-glycine-glycine substrate is cleaved by ACE and react with trinitrobenzesulfonat acid to form 2,4,6-trinitrofenil-glycine-glycine were separated by RP-HPLC.[42,43] In addition, it also has been validated a colorimetric assay for screening ACE inhibitors from plant extracts *in vitro* by Serra *et al.*[44]

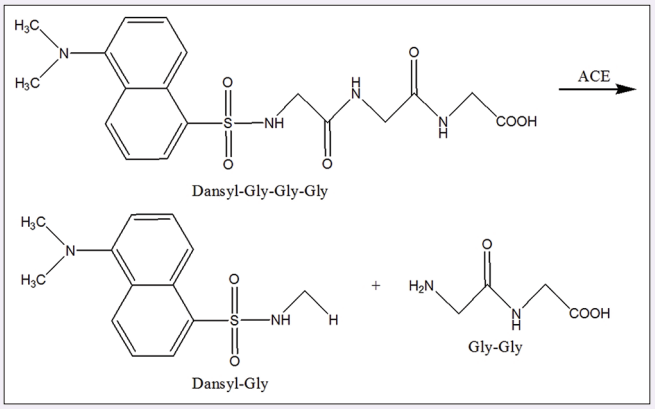

Figure 3: Dansyltriglycyne hydrolysis by angiotensin-converting enzyme

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 benzoyl-[l-14C] glycyl-L-histidyl-L-leucine. The substrate will be cleaved at the glycine-histidine bonding and form benzoyl-[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 benzoyl glycyl-*p*-nitrophenilalanilproline as a substrate then hydrolyzed into *o*-amino benzoyl glycyl as a compound fluorescence, for discontinue enzymatic reaction can be used EDTA.[19] In 2006, Sentandreu and Toldrá 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.[45] 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-WST 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,[29,36] this method also was performed standarization[30]

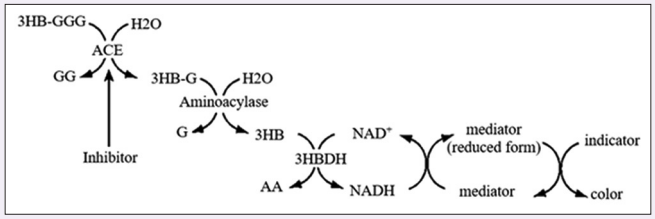

Figure 4: The principle of the assay method of angiotensin-converting enzyme inhibitors activity using angiotensin-converting enzyme kit-Water-Soluble Tetrazolium Salt 1

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				[26]
Abutilon indicum G. Don	Malvaceae	Root	18	
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 viscida W and Arn.	Fabaceae	Root	71	
Sida acuta Burm.	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	
Sedum sarmentosum Bunge	Crassulaceae	Whole plant	24	
Uncaria rhynchophylla Jacks.	Rubiaceae	Root	59	
Species from Chile				
Aristotelia chilensis DC.	Elaeocarpaceae	Bark	36	
Escallonia myrtioides L.	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]
Adiatum 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 elata Jacq.	Asparagaceae	Leaf, bulbs	16	
Drimia robusta Bak.	Asparagaceae	Leaf, bulbs	23	
Dombeya rotundifolia (Hochst.)	Pentapetaceae	Leaf, bark	83	
Ekebergia capensis Sparrm	Meliaceae	Leaf	37	
Hypoxis colchicifolia Bak.	Hypoxidaceae	Leaf, root	37	
Mesembryanthemum sp.	Aizoaceae	Leaf, stem	90	
Protorhus longifolia (Bernh	Anacardiaceae	Leaf	77	
Rhus shirindensis Bak. F.	Anacardiaceae	Leaf, bark	85	
Sclerocarya birrea (A. Rich.)	Anacardiaceae	Leaf	68	
Stangeria eriopus (Kunze) Baill.	Stangeriaceae	Leaf	55	
Tulbaghia tiolacea Harv.	Amaryllidaceae	Leaf, root	72	
Turraea floribunda Hochst.	Meliaceae	leaf	45	
Cecropia glaziovii Snethlage	Cecropiaceae	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...

Table 1: Contd...

Species of plant	Family	Part of plant	ACE-I activity (%)	References
Xylopia frutescens Aubl.	Annonaceae	Seed	3	
Cuphea carthagenensis (Jacq.)	Lythraceae	Leaf	50	
Mansoa hirsuta DC.	Bignoniaceae	Leaf	54	
Tabebuia serratifolia (Vahl)	Bignoniaceae	Stem bark	19	
Clytostoma ramentaceum Bur.	Bignoniaceae	leaf	16	
Musanga cecropioides R.	Urticaceae	Leaf	100	[43]
Crataegus oxyacantha	Rosaceae	Leaf	<40	
Cecropia hololeuca	Urticaceae	Leaf	40	
Cecropia pachystachya Trec.	Urticaceae	Leaf	40-60	
Cecropia glaziovii Sneth.	Urticaceae	Leaf	60-100	
Species of plant	**Family**	**Part of plant**	**ACE-I activity (μM)**	**References**
Olea europaea	Oleaceae	Isolated	26	[40]
Olea lancea	Oleaceae	Isolated	0.33	
Allium sativum L.	Liliaceae	Isolated	3.74	[52]
Species of plant	**Family**	**Part of plant**	**ACE-I activity (μg/ml)**	**References**
Panax ginseng	Araliaceae	Radix	5	[53]
Marrubium radiatum	Lamiaceae	Whole plants	72.8	[54]
Calamintha origanifolia	Lamiaceae	Whole plants	106.2	
Hyssopus officinalis	Lamiaceae	Whole plants	52.0	
Asperula glomerata	Rubiaceae	Whole plants	165.6	
Satureja thymbra	Lamiaceae	Whole plants	289.8	
Salvia acetabulosa	Lamiaceae	Whole plants	52.7	
Periploca laevigata		Root	30	[55]
Species of plant	**Family**	**Part of plant**	**ACE-I activity (μg/ml)**	**References**
Erythroxylum gonocladum (Mart.)	Erythroxylaceae	Arial part	4.53	[56]
Scurulla artopurpurea	Loranthaceae	Herbs	322	[57]
Catharanthus roseus	Apocynaceae	Leaf	402	
Swietenia mahogany	Meliaceae	Seed	774	
Persea americana	Lauraceae	Seed	476	
Oxalis corniculata	Oxalidaceae	Leaf	325	
Phalleria marcocarpa	Thymelaeaceae	Leaf, fruit	102	
Gynura procumbens	Asteraceae	Leaf	227	
Melia azedarach	Meliaceae	Leaf	483	
Hibiscus rosasinensis	Malvaceae	Leaf	271	
Species of plant	**Family**	**Part of plant**	**ACE-I activity (mg/ml)**	**References**
Tamarix hohenackeri Bunge	Tamaricaceae	Arial parts	100	[58]
		Isolated	20	
Apium graveolens	Apiaceae	Whole plants	1.7	[59]
Solanum torvum	Solanaceae	Fruits	1.2	[60]
Species of plant	**Family**	**Part of plant**	**ACE-I activity (μg/ml)**	**References**
Mucuna pruriens	Fabaceae	Seed	38.44	[61]
Sesamum indicum L.	Pedaliaceae	Seed	30.16	[62]
Peperomia pellucida (L.) Kunth	Piperaceae	Herb	7.17	
Nasturtium officinale	Brassicaceae	Herb	15.40	
Peperomia pellucida (L). Kunth	Piperaceae	Ethyl acetate extract herb	3.34	[63]
		Isolated	7.72	

ACE-I: Angiotensin-converting enzyme inhibitory

and validation,[36] 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.[51] Some herbs that have been carried out the screening of activity using the *in vitro* assay method of ACE inhibitors activity as shown in Table 1. Based on the screening results of ACE inhibitors activity from various data sources [Table 1] show that the research of new drug discovery from natural materials is still very limited when compared with the availability of abundant natural resources around us.

The new drug discovery from natural materials are not only limited to the screening phase the activity but till the determining phase of compounds 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 *in silico* assay method[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 (*Apium graveolens*) as antihypertensive and isolation of the junipediol A 8-*O*βD-glucoside (1-βD-glucosyloxy-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-dioxo-3

b, 15a, 24-trihydroxyurs-12-ene-24-O- b-D-glucopyranoside, chrysoeriol-7-O-b-D-apiosylglucoside, apiin, icariside D_2, luteolin-7-O-b-D-glucoside,apigenin-7-O-b-D-glucoside,roseoside,and isofraxidin-b-D-glucoside).[59] Garlic (*Allium sativum*) has been known to dipeptide compounds that play a role as an ACE inhibitor, namely Gly-Phe, Ser-Phe, Gly-Tyr, Ser-Tyr, Asn-Phe, Asn-Tyr, and Phe-Tyr, with the IC_{50}, were 277.9, 130.2, 72.1, 66.3, 46.3, 32.6, and 3.74 μM,[52] respectively. The (E)-2,3-dihydroxycyclopentyl-3-(3',4'-dihydroxyphenyl) acrylate compound was isolated from wild Eggplant (*Solanum torvum*) (IC_{50} of 778 mg/mL).[60] *Suaeda physophora* Pall has isolated a quinoline alkaloids (Suaeda) and flavonoid compounds that also have a potential of ACE inhibitors activity.[65] The astilbin (with IC_{50} of 4.53 μg/mL) were isolated from *Erythroxylum gonocladum* plant.[56] The quercetin isolated from *Peperomia pellucida* L. Kunth herbs with IC_{50} of 7.72 μg/mL.[63] By knowing the active compounds that play a role as an ACE inhibitor, the researchers of natural products are more focused on enrichment, dereplication, and isolation the active compound that can be applied in the *in vivo* assay method and further development.

CONCLUSION

By the discovery of the various assay methods of ACE inhibitors activity. This method is very applicable for testing on natural products so that the researchers can perform quickly, accurately, and simple, 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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