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In vitro ACE inhibitory effects of some Bangladeshi plant extracts

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The angiotensive converting enzyme (ACE) inhibitors lower systemic arteriolar resistance and mean, diastolic, and systolic blood pressures in various hypertensive states. The fall in systemic blood pressure observed in hypertensive individuals treated with an ACE inhibitor results from a reduction of total peripheral resistance in which there seems to be a somewhat variable participation by different vascular beds. Besides causing systemic arteriolar dilatation, ACE inhibitors increase the compliance of large arteries, which contributes to a reduction of systolic pressure [1].

The aim of this study was to screen some Bangladeshi plant extracts for their inhibitory effects on ACE.

Sixteen extracts from thirteen plants were tested *in vitro* against ACE. Of these, seven plant extracts exhibited mild to potent inhibitory activity.

Polyalthea longifolia (Sonn.) Thw. (Annonaceae) when extracted with 80% ethanol, significantly inhibited the ACE and median inhibitory concentration (IC_{50} value) was 0.169 mg/ml. *Semecarpus anacardium* Linn. (Anacardiaceae) extracted with 100% (absolute) ethanol exhibited siginificant ACE inhibition of which the IC_{50} value is 0.172 mg/ml. *Hygrophila auriculata* (Schum.) Heyne. (Acanthaceae) extracted with 80% ethanol exhibited good ACE inhibition of which the IC_{50} value is 0.774 mg/ml. *Hemedesmus indicus* (Linn.) R. Br. (Asclepiadaceae) oil, extracted with 80% ethanol inhibited ACE *in vitro*, but the inhibition was poor. The IC_{50} value is 3.17 mg/ml. The ethanolic extract of the stem bark of *Aegle marmelos* Correa. (Rutaceae) when fractionated with ethylacetate (AcOEt) exhibited ACE inhibition *in vitro*, the IC_{50} value was estimated to be 0.271 mg/ml. The aqueous extract (AQU) from *Shorea rubasta* Gaertn. (Dipterocarpaceae) exhibited good ACE inhibitory activity *in vitro*. The IC_{50} value was estimated to be 0.261 mg/ml.

When the seeds of *Nigella sativa* Linn. (Ranunculaceae) were extracted with 100% (absolute) ethanol, the resultant extract did not inhibit the ACE *in vitro*. But when the ethanolic extract was further fractionated with diethylether then the resultant extract showed ACE inhibition, though the inhibition was very poor and the IC_{50} value for this extract was 5.94 mg/ml.

The 80% ethanolic extract of the stem bark of *Aphanamixis polystachya* Wall. (Melicaceae) (solid and oil), the aqueous extract (AQU) of *Moringa oleifera* Lamk. (Moringaceae), the 100% ethanolic extract from *Pterocarpus santalinus* F. (Papilionaceae), *Adhatoda vasica* Nees. (Acanthaceae), *Artocarpus heterophyllus* Lamk. (Urticaceae), *Aegle marmelos* Correa. (Rutaceae) and *Ocimum sanctum* Linn. (Labiteae) were devoid of any ACE inhibitory activities *in vitro*.

Additional studies, e.g., the compound(s) responsible for the *in vitro* ACE inhibitory activities are in progress. We are now purifying, identifying and studying their *in vivo* antihypertensive activity in spontaneously hypertensive (SHR) rats. For these purposes we choose the plant extracts which exhibited highest inhibitory activities.

Experimental

1. Plant materials and extraction

The dried plant materials, stem bark of *Aphanamixis polystachya*, stem bark of *Polyalthia longifolia*, nuts of *Semecarpus anacardium*, seeds of *Hygrophila auriculata*, root of *Hemidesmus indicus*, stem bark of *Aegle marmelos*, seeds of *Nigella sativa*, stem bark of *Moringa oleifera*, stem bark of *Shorea rubasta*, wood of *Pterocarpus santalinus*, stem bark of *Adhatoda vasica*, seeds of *Artocarpus heterophylus*, and leaves of *Ocimum sanctum*, were extracted with different solvents mentioned in the Table 1.

2. Chemicals and reagents

Purified rabbit lung ACE (angiotensin I-converting enzyme) was purchesed from Sigma (MO, USA). Hippuryl-L-histidyl-L-leucine (Hip-His-Leu) as a synthetic ACE substrate was from Peptide Institute (Osaka, Japan). All other reagents used in this study were purchased from Nacalai Tesque (Kyoto, Japan). For the extraction purposes of the plant materials all the solvents were purchased from E. Merck, Germany.

3. Assay for ACE inhibitory activity

ACE inhibitoy activity was measured by a modification of the method of Lieberman as described by Yamamoto et al. [2]. Briefly, 25 µl of ACE inhibitor and 50 µl of 12.5 mM Hip-His-Leu in a borate buffer (pH 8.3) containing 200 mM NaCl were incubated with 50 µl of ACE (25 mU/ml) at 37 °C for 1 h. The reaction ws stopped by adding 125 µl of 0.5 M HCl, followed by the addition of 750 µl of ethyl acetate (AcOEt). After the extraction of hippuric acid with AcOEt, 250 µl of the AcOEt layer was dried under reduced pressure, and redissolved in 1.5 ml of 300 mM NaCl solution. After mixing, the absorbance of the produced hippuric acid at 288 nm was measured with a Shimadzu UV-1200 spectrophotometer (Kyoto, Japan). The ACE inhibitor concentration required to inhibit 50% of the ACE activity under the assayed conditions was defined as the IC_{50} value.

Table 1: *In vitro* ACE inhibitory activity of Bangladeshi plant extracts (IC_{50} in mg/ml)

Plants (Family)	Solvent used for Extraction	IC_{50} (mg/ml)
Aphanamixis polystachya Wall. (Meliaceae) (solid)	80% EtOH	N.I.*
Aphanamixis polystachya Wall. (Meliaceae) (liquid)	80% EtOH	N.I.*
Polyalthea longifolia (Sonn.) Thw. (Annonaceae)	80% EtOH	0.169
Semecarpus anacardium Linn. (Anacardiaceae)	100% EtOH	0.172
Hygrophila auriculata (Schum.) Heyne. (Acanthaceae)	80% EtOH	0.774
Hemedesmus indicus (Linn.) R. Br. (Asclepiadaceae) (oil)	80% EtOH	3.17
Aegle marmelos Correa. (Rutaceae)	100% EtOH	N.I.*
Nigella sativa Linn. (Ranunculaceae)	100% ETHER	5.94
Moringa oleifera Lamk. (Moringaceae)	AQU[a]	N.I.*
Shorea rubasta Gaertn. (Dipterocarpaceae)	AQU[a]	0.261
Nigella sativa Linn. (Ranunculaceae)	100% EtOH	N.I.*
Aegle marmelos Correa. (Rutaceae)	AcOEt	0.271
Pterocarpus santalinus F. (Papilionaceae)	100% EtOH	N.I.*
Adhatoda vasica Nees. (Acanthaceae)	100% EtOH	N.I.*
Artocarpus heterophyllus Lamk. (Urticaceae)	100% EtOH	N.I.*
Ocimum sanctum Linn. (Labiteae)	100% EtOH	N.I.*

* N.I. = No inhibition; [a]AQU = aqueous extract; AcOEt = Ethyl acetate.

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The effect of *Premna integrifolia* Linn. (Verbenaceae) on blood glucose in streptozotocin induced type 1 and type 2 diabetic rats

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A total of more than 400 plants display hypoglycemic effects, but few of them have been investigated scientifically [1]. *Premna integrifolia* Linn. syn. *P. obtusifolia* R.Br. (Verbenaceae) grows commonly in the Indian and the Andaman coasts. The decoction of the root is a cordial, stomachic, laxative, and useful in liver disorders and diabetes [2, 3]. Some traditional healers use this as hypoglycemic agent. The plant possesses hypoglycemic action in rats [4, 5]. The present study investigated the effect of powder and alcoholic extract of the stem-bark of *Premna integrifolia* L. on blood glucose levels in streptozotocin induced Type 1 and Type 2 model rats in different prandial states.

The powder and the alcoholic extract did not show any hypoglycemic activity in Type 1 rats in fasting and 30 min before the glucose load state. When the drug was administered simultaneously with glucose, the powder did not alter blood glucose concentration, but the extract significantly ($p < 0.05$) opposed the rise of blood glucose in Type 1 rats (Table 1).

In Type 2 rats, a rise ($p < 0.05$) in the glucose level was evident in the powder group at the fasting state, revealing the possibility of negative action on β cell or enhancing gluconeogenesis at the fasting state. The extract had no effect at this fasting state. Both the powder and the extract did not alter the blood glucose level in Type 2 rats, when they were fed 30 min before glucose load. The extract group, when fed simultaneously with glucose, improved glucose tolerance ($p < 0.02$) in Type 2 rats (Table 2).

We conclude that *Premna integrifolia* L. powder did not show any hypoglycemic effects, but the extract showed a mild antihyperglycemic effect under our experimental conditions due to the possible effects of delaying intestinal glucose absorption in the GI tract or due to the increase of hepatic or peripheral glucose disposal.

Table 1: Effect of *Premna integrifolia* L. on Type 1 diabetic rats

Group	Serum glucose level mmol/l (p value)			
Fasting	0 min	60 min		120 min
Control (n = 10)	25.50 ± 1.22	23.70 ± 1.20		22.88 ± 1.24
Powder (n = 9)	26.40 ± 1.71 (0.669)	24.87 ± 1.24 (0.509)		22.40 ± 1.43 (0.796)
Extract (n = 9)	26.91 ± 1.11 (0.411)	25.79 ± 0.84 (0.182)		24.49 ± 0.77 (0.298)
30 min before glucose load	0 min	60 min	105 min	iobv
Control (n = 6)	27.44 ± 1.38	33.70 ± 1.99	31.82 ± 1.70	10.65 ± 1.70
Powder (n = 6)	26.89 ± 3.20 (0.878)	33.48 ± 1.58 (0.933)	30.48 ± 2.13 (0.634)	10.19 ± 4.57 (0.927)
Extract (n = 7)	27.40 ± 1.94 (0.988)	35.10 ± 2.37 (0.665)	32.26 ± 1.12 (0.828)	12.56 ± 2.17 (0.512)
Simultaneously with glucose load	0 min	30 min	75 min	iobv
Control (n = 7)	22.55 ± 0.88	30.43 ± 1.67	30.47 ± 1.83	15.78 ± 3.44
Powder (n = 6)	22.21 ± 1.05 (0.805)	32.66 ± 1.56 (0.356)	31.46 ± 1.96 (0.720)	19.70 ± 1.62 (0.352)
Extract (n = 8)	26.49 ± 1.69 (0.069)	30.20 ± 1.41 (0.919)	29.53 ± 1.44 (0.691)	6.74 ± 2.19 (**0.040**)

n = number of rats
iobv = sum of increments over basal value