

Angiotensin Converting Enzyme (ACE) : Inhibition of Rabbit Lungs ACE in Vitro by *Nardostacys Jatamansi*

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ABSTRACT

In renin-angiotensin-Aldosterone-system, renin produced from juxtaglomerular apparatus of kidney. Produced renin, convert angiotensin to inactive angiotensin-I. With the help of angiotensin converting enzyme, angiotensin-I converted into angiotensin-II which leads to hypertension. In this study methanol, ethyl acetate and petroleum ether extract of *Nardostachys jatamansi* was used to inhibit ACE. In vitro assay was based on release of hippuric acid from substrate Hippuric-Histidyle-Leucine by ACE and measured spectrophotometrically at 228 nm. ACE from rabbit lungs (sigma Aldrich) was inhibited by methanol, petroleum ether and ethyl acetate extract of *Nardostacys jatamansi* and higher percentage of inhibition was observed in methanolic extract that confirmed by standard drug inhibitor like Captopril. HPTLC of plant extract was carried out using Toluene: Ethyl acetate, 9.3:0.7 solvent system. Presence of different phytoconstituents was observed in HPTLC finger prints. These different phytoconstituents present in the plant help to inhibit ACE. This result support traditional use of plant as antihypertensive.

Keywords : Angiotensin Converting Enzyme, *Nardostacys Jatamansi*, Vitro, HPTLC, Plant materials

I. INTRODUCTION

Hypertension is a worldwide major risk factor for cardiovascular disease. Hypertension also causes stroke, renal impairment, arteriosclerosis and end stage renal disease. Hypertension is a leading member of the group of so called “non – communicable disease” (NCD) and leading contributory cause of death worldwide (Maghrani M et al., 2005). In the treatment of hypertension, inhibition of angiotensin converting enzyme (ACE) is established as one of the modern therapeutic approach (Adeneye AA et al., 2006). The renin-angiotensin –aldosterone system (RASS) has an important contribution in the maintenance of vascular tone and involved in controlling blood pressure (Saputri et al., 2015). In renin –angiotensin – aldosterone system, renin produced from juxtaglomerular apparatus of kidney. Which split angiotensin into inactive angiotensin –I. With the

help of angiotensin converting enzyme, inactive angiotensin – I converted to active octapeptide, angiotensin – II. Produced angiotensin – II effects both peripheral and central vascular system and also promotes sodium and water retention that cause an increase in renin production in kidney (William LAD et al., 1997). These leads to blood pressure, hypertension cardiac failure, myocardial infarction etc .So many synthetic angiotensin converting enzyme inhibitors are present in the market like Captopril, Enalapril, Lisinopril. However synthetic drugs have many side effects like cough, skin problems, nausea, Hyperkalemia, fatigue, dizziness, hypotension, renal impairment etc (Pool MD and Postma DS, 1991). Therefore, for safe and economical use, identifying natural medicinal source as ACE inhibitors has increased. Some of plants were also used as traditional medicine since a long time ago to decrease blood pressure. But the scientific data is limited.

Nardostachys jatamansi is commonly known as musk root. It is found mostly in indigenous region of Himalayas, in India. Experimentally, the plant is reported as anticonvulsant, antioxidant and lipid peroxidation activity in mice. Also, the essential oil isolated from the plant had showed the fungicidal activity (Sarbhoy AK et al., 1978).in Ayurvedic, roots and rhizome of *Nardostachys jatamansi* used to treat hysteria, epilepsy and convulsion (Bagchi A et al., 1991).

II. MATERIAL AND METHOD

Plant collection: Plant materials were collected from bapalal garden, bioscience department, Veer Narmad South Gujarat University, Surat.

Materials: Materials for extraction and fractionation including petroleum ether, ethyl acetate, and methanol, NaOH, distilled water, hydrochloric acid. ACE from rabbit lungs,(purified ACE) hippuric acid, hippuric-histidyle-l-leucine, (sigma) Nacl, tris HCL buffer.

Extract preparation: Plants were washed in water; dried at 40c and powdered each material (100g) was macerated with petroleum ether. Maceration repeated again with the same solvent until the filtrate gives clear maceration results. Filtrate was concentrated using rotary vacuum evaporator at a temperature of approximately 50c to obtain petroleum ether extract. Do same for ethyl acetate, methanol.

Preliminary phytochemical screening: The plant may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannic acid, saponins, flavonoids, etc. The preliminary phytochemical screening of all the extract of *Nardostachys jatamansi* was carried out as per the standard procedure (Khandelwal K.R, 2007).

Angiotensin converting enzyme inhibition assay: ACE inhibition activity was determined by modified

method of (Cushman and Cheung 1971). 50µl of ACE (25µg/ml) and 50µl of plant extract sample at different concentration was incubated for 10 min. for 37. c. Then add 150µl of substrate solution (8.3mM HHL in 50mM tris HCL buffer containing 0.5 M Nacl at PH 8.3) and incubated again at 37.c for 30 min. The reaction was terminated by adding 250µl of 1.0 M HCL. The produced hippuric acid was extracted with 1ml of ethyl acetate. After centrifugation (800g, 15 min) 0.8ml of upper layer was transfer into a test tube and evaporated at room temperature for 2 hr in vacuum. Hippuric acid was dissolved in 1ml of D.W finally the absorbance was measured at 228nm using UV-spectrophotometer. ACE inhibition test was performed on three samples: positive control, negative control and fraction prepared from sample plants. Captopril was used as positive control.

Calculation: For all tests, the inhibition assay was performed in triplicate and ace inhibition percentage calculated as follow:

$$\text{INHIBITION} = 100 \times (1 - A/C)$$

A= absorbance test – absorbance black

C= absorbance negative control

High Performance Thin Layer Chromatography: HPTLC is the fastest, automated and sophisticated form of TLC (Srivastava M, 2011). In HPTLC, Precoted silica gel plate G 60 F254 was used for the application of sample. Solvent system of HPTLC was optimized by performing TLC. 10µl sample of different extract was applied on 10×10 cm size Precoted silica gel having 250µm thickness. Toluene: Ethyl acetate, 9.3:0.7 ratio solvent systems was used. Detection was done under 254nm and 366nm wavelength and scanning was carried out by CAMAG TLC scanner and densitometry evaluation with WINCATS software.

Result:

Extraction: The plant material was extracted by maceration method using three different solvents,

methanol, ethyl acetate, petroleum ether. Final extract was obtained under vacuum evaporation. Among these three different solvent extract, highest yield was obtained in methanolic extract of *Nardostachys jatamansi* (Table 1).

Table 1. Total percentage yield of plant extract

Plant Name	Percentage yield (W/W)		
	Methanol	Ethyl acetate	Petroleum ether
<i>N. jatamansi</i>	2.91	1.65	0.50

The yield was calculated by comparing with initial weight of the powdered plant material

Phytochemical screening of extract: The results of the phytochemical screening of the methanol, ethyl acetate, and petroleum ether solvent extract contain flavonoids, saponins, and alkaloids. (Table 2)

Table 2. Summary of phytochemical analysis of plant extract

<i>N. jatamansi</i>	Methanol	Ethyl acetate	Petroleum ether
Alkaloids	+	+	-
Flavonoids	+	+	+
Phenols	+	-	+
Saponins	-	+	-

Angiotensin converting enzyme inhibition assay: Conducting the inhibitory effects of methanol, ethyl acetate, and petroleum ether plant solvent extracts of *Nardostachys jatamansi* on angiotensin converting enzyme. The ACE inhibitory activities of plants were represented as percentage inhibition by the different solvent extract. The result shown that all three solvent extract gives ACE inhibition but the higher inhibition

was done by methanolic extract of *Nardostachys jatamansi*. ACE inhibition percentage were shown in figure 1 along with Captopril as standard.

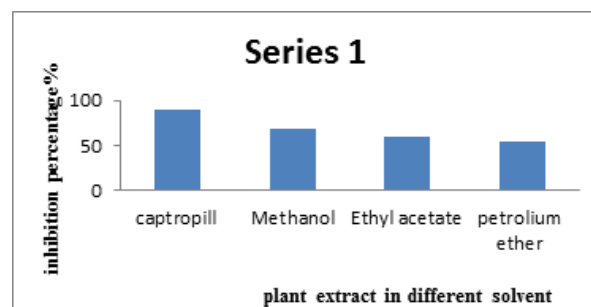
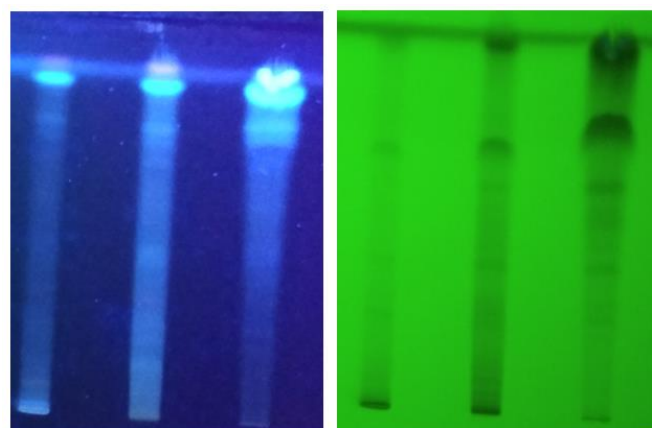


Figure 1. Extract of *Nardostachys jatamansi* in Methanol, Ethyl acetate and Petroleum ether solvent shows ACE inhibition along with Captopril as standard.

High performance thin layer chromatography: The results of HPTLC fingerprints was scanned at 366 and 254 nm wavelength by CAMMAG for plant extract of *N. jatamansi* in methanol, ethyl acetate and petroleum ether solvent show polyvalent phytoconstituents (figure 2).



HPTLC plate at 366 nm

HPTLC plate at 254 nm

Track 1 Methanolic extract Track 2 Ethyl Acetate extract Track 3 Petroleum Ether extract

Figure 2. HPTLC finger print of *Nardostachys jatamansi*

III. DISCUSSION

Extraction: Rhizome of *N. jatamansi* plant was extracted by maceration using methanol, ethyl acetate,

and petroleum ether solvents. Crude extract yield were calculated based on dry weight. Percentage yield was between 0.50-2.91 as show in Table 1. But the highest yield was obtained in methanolic extract of plant material.

Phytochemical screening: *Nardostachys jatamansi* plant material extracted with methanol, ethyl acetate and petroleum ether was investigated for different phytochemical constitution like flavonoids, alkaloids, phenols and saponins etc as results show in Table 2. The biological activities of medicinal plants such as hypoglycaemia, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic etc (Negi JS et al., 2011). ACE inhibition activity was mostly due to the flavonoids content present in the plants. Flavonoids were given in vitro activity via generation of chelate complex within the active part of ACE (Balasuriya BWN et al., 2011). Free hydroxyl group of phenolic compounds are also structural moieties to chelate zinc ion that present in ACE in the form of zinc containing dipeptide hydrolyse (Ojeda D et al.,2010). This result suggest that polyvalent phytochemical present in this plant may responsible for inhibition of angiotensin converting enzyme.

Angiotensin converting enzyme inhibition assay: Recent year has witnessed a rising interest in plant extract with different phytochemical constituents with regards to their potential for cardiovascular protection and antihypertention activity (Nawaji N N et al, .2016).the ace inhibitory activity of N. jatamansi plant in methanol, ethyl acetate, and petroleum ether crude extract was evaluated in vitro at different concentration. The inhibition percentage of ace activity by different concentration of the extract was tested and compared with standard Captopril drug. By conducting in vitro ace inhibition assay, maximum inhibition was observed in methanolic extract (68.36%) of *Nardostachys jatamansi* which is compared to standard drug (89.36%).

High performance liquid chromatography: The HPTLC fingerprint analysis confirmed that the plant extract prepared in different solvent possess many phytoconstituents. The study of HPTLC fingerprints report different phytochemical that is suitable to confirm the identity of medicinal plant raw material. These different phytochemical considered to be a natural antihypertensive and may be a promising candidate for the treatment of other metabolic disorder.

IV. CONCLUSION

This study indicates that *Nardostachys jatamansi* plant shows ACE inhibitory effect in three different solvent extract. It gives good result in methanolic extract of the plant that used to treat hypertension. This plant may be the alternative for the ACE inhibitors. Further studies are required for the identification of active compounds that gives maximum inhibition against ACE as compared to standard synthetic drugs.

V. REFERENCES

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