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ANTIHYPERTENSIVE ACTIVITY GUIDED-FRACTIONATION OF *NARDOSTACHYS JATAMANSI*

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ABSTRACT: The present study aimed to examine the anti-hypertensive constituents present in *Nardostachys jatamansi*. Crude solvent extracts (dichloromethane; ethyl acetate; methanol, and water) of this plant rhizomes based on the difference in polarity were prepared and tested for inhibition of angiotensin-converting enzyme (ACE). Preliminary phytochemical analysis showed bioactive compounds such as alkaloids, flavonoids, carbohydrates, triterpenoid, glycosides, saponin, and tannins in different extracts. Ethyl Acetate extract has shown better results with 93% inhibition at 100 µg concentration as compared to other solvent extracts. Fractionation of ethyl acetate extract over silica gel column chromatography with hexane and ethyl acetate under gradient elution resulted in six fractions. However, on purification of the ethyl acetate extract, ACE inhibition activity diminishes with only 19.03 to 47.35% inhibition for the purified fractions. *Nardostachys jatamansi* can be used to treat hypertension-related disorders, and ethyl acetate extract can be further investigated to identify potent molecules that work in a synergistic combination.

INTRODUCTION: *Nardostachys jatamansi* (family Valerianaceae) is a perennial herb found in Alpine Himalayas. It is a reputed Ayurvedic herb and have demonstrated hypotensive, hypolipidemic, antimicrobial, antiarrhythmic, hepatoprotective, *etc*. [1-8] Ethanol extract of the roots of *N. jatamansi* DC was studied for its anticonvulsant activity and neurotoxicity demonstrated a significant increase in the seizure threshold by *N. jatamansi* DC root extract against maximal electroshock seizure (MES) model as indicated by a decrease in the extension/flexion ratio [9].

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Chemical examination of this plant revealed the presence of terpenoid ester, nardostachysin N, volatile, non-volatile constituents, sesquiterpenes coumarins, lignans, neolignans, alkaloids. Acacin, Ursolic acid, Octacosanol, Kanshone A, Nardosinonediol, Nardosinone, Aristolen-9beta-ol, Oleanolic acid, Beta-sitosterol are chemical constituents isolated from this plant [10-12]. The Discovery of the angiotensin-converting enzyme (ACE) leads to the drug discovery of anti-hypertensive drugs. The ACE converts angiotensin decapeptide inactive form into active octapeptide angiotensin II in the kidneys, especially in the renin-angiotensin-aldosterone system [13]. Captopril, Enalapril, Zofenopril, Ramipril, Fosinopril, Lisinopril, and SQ 29852 are the well-known ACE inhibitors [13]. Generally, Captopril is used as a positive control in most of the research study owing to its free radical scavenger activity that is highly relevant as an ACE inhibitor [13].

Considering the above facts, the present study is aimed to conduct phytochemical screening and to examine the anti-hypertensive constituents present in rhizomes of *Nardostachys jatamansi*.

MATERIALS AND METHODS:

Plant Material: *Nardostachys jatamansi* rhizomes were collected in the month of June 2016 from High Altitude Plant Physiology Research Centre, Srinagar [Gharwal] Uttarkhand, India. Rhizome was authenticated by comparing with the Herbarium (No. 2016/52) maintained at Department of Botany, J.S.S College Belagavi, Karnataka, India.

Extraction: The rhizomes were coarsely powdered in a pulverize with sieve # 16 in K.L.E Ayurveda Pharmacy, Shahapur, Belagavi, Karnataka, India. 100 g of each powdered material was subjected to Soxhlet extraction by using 400 mL of different solvents *viz.* dichloromethane, ethyl acetate, methanol, and water individually.

The extracted solution was dried individually under vacuum using a rotary vacuum evaporator and preserved in different amber-colored bottles.

Phytochemical Screening: The preliminary phyto-chemical screening was performed to characterize the various phytochemicals present in *Nardostachys jatamansi* rhizomes [14]. For instance, for the determination of alkaloids, Mayer's test, Hager's, and Dragendroff's test were performed.

Ferric chloride test was followed to identify tannins and phenolics. For the presence of glycosides, a Legal test was performed. An alkaline test was implemented to check flavonoids. Ninhydrin and Biuret test were followed to detect proteins. Steroids were checked through Salkowaski test. To test the presence of carbohydrates, Biuretic and Fehling's tests were employed.

Fractionation and Purification: Ethyl acetate extract was fractioned over silica gel column chromatography (60-120 mesh) under gradient elution of hexane and ethyl acetate (500 mL each) and methanol (500 mL) to provide dried fractions:

Fr.1 (10% ethyl acetate in hexane); Fr. 2 (25% ethyl acetate in hexane); Fr. 3 (50% ethyl acetate in hexane); Fr. 4 (75% ethyl acetate in hexane); Fr. 5 (100% ethyl acetate) and Fr. 6 (100% methanol).

Angiotensin-Converting Enzyme [ACE] Inhibitory Assay: Solvent extracts were subjected for anti-hypertensive activity by angiotensin-converting enzyme [ACE] inhibitory method with different concentrations of extract in comparison with a standard, captopril. ACE inhibition activity was carried out at BioGenics Labs, Hubli, Karnataka, India. ACE inhibition activity measured by a UV spectrophotometer based on the rate of formation of hippuric acid from hippuryl-L-histidyl-L-leusine (hhl) catalysed by ACE [13].

Extracts were tested at 10, 50, and 100 µg dissolved in assay buffer (10mM) HEPES buffer containing 0.3 M NaCl and 10 µM zinc sulphate containing 20 µL kidney cortex plasma membranes (ACE enzyme source) and hippuryl-L-histidyl-L-leusine (hhl) as substrate. Briefly, the tested extracts are incubated at 37 °C for 10 min. Then the substrate is added, which makes a final reaction volume of 50 µL and incubated for 45 min at 37 °C. The reaction is terminated by the addition of 1M HCl. The yellow color is developed by the addition of 100 µL of pyridine and 50 µL of benzene sulphonyl chloride. The yellow color that formed is measured at 410 nm in an ELISA plate reader (iMark, BIORAD). Extracts with an inhibitory potential block the substrate availability to the enzyme and thereby cause enzyme inhibition leading to no formation of yellow color. The inhibition is represented in the form of percentage over control. Captopril, a standard, was tested at a concentration of 10, 15, and 25 nM. The rate of ACE inhibition was calculated by the formula,

% Inhibition = Δ Control absorption – Δ Sample absorption / Δ Sample absorption

RESULTS AND DISCUSSION: Preliminary phytochemical analysis showed that the occurrence of bioactive compounds such as alkaloids, flavonoids, carbohydrates, triterpenoid, glycosides, saponin, and tannins in different extracts **Table 1**.

ACE inhibition at 100 µg concentration follows the order: Ethyl acetate Extract (93.23%) > Water Extract (74.23%) > Dichloromethane Extract (67.03%) > Methanol Extract (56.77%). Among all the tested extracts, ethyl acetate extract has shown better result with 93% inhibition at 100 µg concentration as compared to other solvent

extracts. Captopril, a standard, was found to inhibit 85.37 % ACE at 25 nM.

Based on the ACE inhibition results, EtOAc extract was fractioned into six fractions: Fr. 1 (10% ethyl acetate in hexane); Fr. 2 (25% ethyl acetate in hexane); Fr. 3 (50% ethyl acetate in hexane); Fr. 4 (75% ethyl acetate in hexane); Fr. 5 (100% ethyl acetate) and Fr. 6 (100% methanol) **Fig. 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF EXTRACTS OF *NARDOSTACHYS JATAMANSI* RHIZOMES

S. no.	Phytochemical Test	Dichloromethane	Ethyl Acetate	Methanol	Water
1	Alkaloids	+ ve	+ ve	+ ve	- ve
2	Tannins	+ ve	+ ve	+ ve	- ve
3	Saponins	+ ve	+ ve	+ ve	+ ve
4	Flavonoids	+ ve	+ ve	+ ve	+ ve
5	Carbohydrates	+ ve	+ ve	+ ve	+ ve
6	Glycosides	+ ve	+ ve	+ ve	+ ve
7	Steroids	+ ve	+ ve	+ ve	- ve

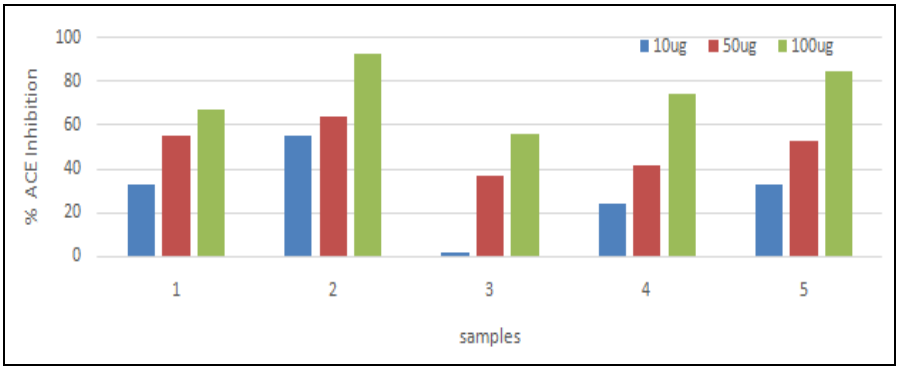

FIG. 1: ACE INHIBITION ACTIVITY OF EXTRACTS OF NARDOSTACHYS JATAMANSI RHIZOMES. Sample 1-Dichloromethane Extract (10, 50 and 100 µg); Sample 2-Ethyl acetate Extract (10, 50 and 100 µg); Sample 3-Methanol Extract (10, 50 and 100 µg); Sample 4-Water Extract (10, 50 and 100 µg); Sample 5-Captopril (10, 15 and 25 nM) (From Left to Right)

Six fractions of ethyl acetate extract were submitted for the ACE inhibition to identify the active constituent(s). Results revealed that Fr. 5 had shown significant inhibition at 100 µg but no activity found at lower concentrations. Fr. 4 has shown a similar tendency, but inhibition at 100 µg is less than Fr. 5. Fr. 3 has shown elevation in inhibitory activity at 50 and 100 µg. The sample has shown little activity at 100 µg whereas Fr. 1, 2 have not shown any inhibition at all concentrations screened, while captopril, a standard, was found to inhibit 85.37% ACE at 25 nM **Fig. 2**.

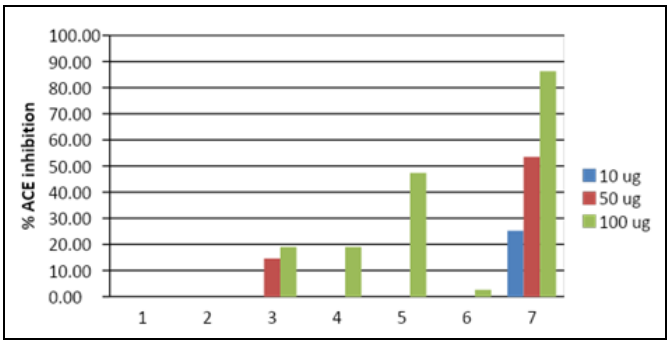

FIG. 2: ACE INHIBITION ACTIVITY OF FRACTIONS OF ETHYL ACETATE EXTRACT OF *NARDOSTACHYS JATAMANSI* RHIZOMES. Fr.1 (10, 50 and 100 µg); Fr. 2 (10, 50 and 100 µg); Fr. 3 (10, 50 and 100 µg); Fr. 4 (10, 50 and 100 µg); Fr. 5 (10, 50 and 100 µg); Fr. 6 (10, 50 and 100 µg); Captopril (10, 15 and 25 nM) (From Left to Right)

It is clearly evident from the ACE inhibition activity results **Fig. 1** & **2** of fractions of ethyl acetate extract of *Nardostachys jatamansi* rhizomes that there is a synergistic activity of constituents towards ACE inhibition. Among the tested extracts, ethyl acetate extract has shown prominent 93% inhibition at 100 µg concentration. However, on purification of the ethyl acetate extract, ACE inhibition activity diminishes with only 19.03 to 47.35% inhibition for the purified fractions (Fr.1 to Fr.6).

CONCLUSION: The present study was designed to examine the anti-hypertensive constituents present in *Nardostachys jatamansi*. Among all the tested extracts, ethyl acetate extract was found to be active against ACE inhibition. However, ACE inhibition was found to diminishes significantly on further purification of ethyl acetate extract. This suggested that potent molecules are present in ethyl acetate that works in synergistic combination and

justify the use of *Nardostachys jatamansi*for treatment of hypertension-related disorders.

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CONFLICTS OF INTEREST: The authors declare no competing financial interest exists.

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