# Seasonal environmental variations on physicochemical properties of Justicia adhatoda- An Indian Medicinal Plant.

**Gautam Sadashiv Palshikar[1]*, Vivek Subhash Tarate[2], Kishor Vasant Otari[3], P. Shanmuga Pandiyan[4]**

## Abstract

Plants live on a planet with days and seasons, and that affects their phytoconstituents. Challenge is, availability of active principles in medicinal plants change by seasonal fluctuations, so their dose pattern for therapeutic efficacy also gets influenced. Seasonal impact show changes in important constituents like polyphenol, flavonoids, glycosides, alkaloids, essential oil etc. Late summer is the best collection time for essential oil component. Winter and rainy are best season for other secondary metabolites. The selected plant i.e. Justicia adhatoda, belongs to alkaloidal antidiabetic category. It was evaluated for pharmacognostic study which includes macroscopic and microscopic evaluation, determination of physicochemical parameters in a systematic way. HPTLC fingerprinting for vasicine was done. Study was performed for plant material with three different seasons and best results were analysed. It showed correct taxonomy with specific morphological, microscopical and physico-chemical parameters which is helpful for the standardization of drugs. Extracts showed presence of alkaloids, terpenes, flavonoids, steroids, phenolics, saponin and carbohydrate. HPTLC fingerprinting confirmed the presence of vasicine in the plant extracts. Seasonal variations occur in plant constituent shows best collection period. Current research aims to focus on best possible season for the harvesting of some pharmaceutically important plant materials.



**Key Words:** Secondary metabolites, Herbal medicines, alkaloids, antidiabetic, seasonal variations

**DOI Number: 10.14704/nq.2022.20.8.NQ44249** **NeuroQuantology 2022; 20(8):2284-2290**

## Introduction

Justicia addhatoda L. an Indian medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years and they continue to be an important therapeutic aid for alleviating the ailments of human kind. In India, it is estimated that 80% of population depends on plants to therapy themselves, of those about 60% populace use medicinal plants habitually to battle certain ailments and almost 40% human use such plants in pharmaceutical industries1. The World Health Organization (WHO) has outlined herbal medicine as culminated labelled medicinal products that incorporate lively ingredients as aerial or underground accessories of plants. Of the 2,50,000 higher plant species on earth, more than 80000 species are reported to have at least some medicinal value2. Since ages, humans have relied on nature for their basic needs for the production of foodstuff, shelters, clothing, means of transportation, fertilizers, flavors, and fragrances, and medicines. Plants have formed the basis of sophisticated traditional systems of medicine that have been in existence for thousands of years and continue to provide humankind with new remedies3. The history of herbal medication is equally old as human history. Most of these plant-derived drugs were originally identified through the subject of traditional remedies and folk knowledge of indigenous people and some of these could not be substituted despite the tremendous progress in synthetic chemistry.

**Corresponding author:** Gautam Palshikar
**Address:** [1,4]PRIST Deemed to be University, Thanjavur, [2,3]N.E.S.'s Navsahyadri Institute of Pharmacy, Naigaon, Pune, Maharashtra, India
E-mail: gautampalshikar@rediffmail.com

Modern medicines and herbal medicines are complimentary being used in areas for health care program in various developing countries including India4. In the present scenario, the demand for herbal products is growing exponentially throughout the globe and major pharmaceutical companies are currently carrying on extensive research on plant materials for their potential medicinal value5,6. The need of new therapies for glycemic control is the fact that existing treatments have limitations because of their side effects7.

The herbal extracts which are effective in lowering blood glucose level with minimal or no side effects are known to be used as antidiabetic remedies8. Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues9. According to International Diabetes Federation (IDF), the number of individuals with diabetes in 2011 crossed 366 million, with an estimated 4.6 million deaths each year10. According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care11.The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India12. There are about 800 plants which have been reported to show antidiabetic potential. A wide collection of plant-derived active principles representing numerous bioactive compounds have established their role for possible use in the treatment of diabetes13. A chromatographic fingerprint of a Herbal Medicine is a chromatographic pattern of the extract of certain common chemical components of pharmacologically active and or chemical constituents. This chromatographic contour should be highlighted by the essential attributions of reliability and fuzziness or similarity and differences so as to chemically represent the herbal medicine explored14. Phytochemical changes due to various seasons were studied by performing HPTLC densitometric quantification. Microscopic variation observed in the quantity of cell inclusions, number of fibers and wall thickness of lignified cells. Physicochemical parameters also showed variation15.

## Material and methods

### Collection and Identification of Plant material

The plant material was collected in every month of the year 2020-21, i.e., in the Rainy season (June, July, August, September), Winter (October, November, December, January) and Summer (February, March, April, May), from places of different altitude i.e. Low (560 meters), medium (920 meters) and high (1,312 meters), twice i.e. morning and evening. Authentication was done by Taxonomist of the Botanical Survey of India, Pune. A voucher specimen (No. BSI/WRC/100-1/Tech./2019/02) was deposited in the Herbarium of Botanical Survey of India, Pune.

### Assessment of quality of plant materials

The plant materials were assessed as per WHO guideline.

Macroscopic evaluation- Fresh plant parts were subjected to color, odor and taste, determination of shape, size, surface characteristics and appearance.

Microscopic evaluation- For microscopical examinations, free hand sections of the fresh leaf were cut, cleared with chloral hydrate solution and water, and stained with a drop of hydrochloric acid and phloroglucinol. Photomicrographic images were taken by using Trino CXR camera.



### Quantitative microscopy

Leaves were subjected to quantitative microscopy for the following values using reported method.- Stomatal number, Stomatal index, Palisade ratio, Vein islet number, Vein termination number,

### Proximate analysis

Proximate analysis of powdered plant material was carried out using reported methods such as Foreign organic matter, Loss on drying, Total ash, Water soluble ash, Acid insoluble ash, Sulphated Ash, Water soluble extractives, Alcohol soluble extractives, Ether soluble Extractive value

### Phytochemical screening

The air dried powder (1 Kg) of plant was extracted in soxhlet apparatus with solvents of increasing polarity as follows: Petroleum ether, Chloroform, Ethyl acetate, Ethanol.

Each time before extracting with the next solvent, the material was dried. All the extracts were concentrated by distilling the solvent and the extracts were dried on water bath. Then consistency, color, appearance of the extracts and their percentage yield were noted.

Establishment of qualitative phytoprofile of successive solvent extracts. (chemical tests)

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins and phytosterols using reported methods.

## HPTLC Analysis

**Table 1: Mobile phase used for HPTLC analysis**

| Plant Name | Phyto Consti. | Std. Area | Mob. Phase | λ max (nm) |
|---|---|---|---|---|
| J. adhatoda | Vasicine | 5053.5 AU | Ethyl acetate: Methanol: Ammonia (8:2:0.2) | 254 |

## Results and Discussion

**Table 2: Quantitative microscopy of J. adhatoda L.. leaf**

| S. No | Parameter | Summer | Rainy | Winter |
|---|---|---|---|---|
| 1 | St. number | 6.4 | 7.2 | 6.7 |
| 2 | St. Index | 11.4 | 11.7 | 12.5 |
| 3 | Palisade ratio | 1.3 | 1.3 | 1.4 |
| 4 | Vein islet no | 23 | 25 | 23 |
| 5 | Vein termi no. | 35 | 36 | 36 |



## Proximate Analysis

**Table 3: Proximate Analysis- of J. adhatoda L. leaf**

| S. No | Parameter (%) | Summer | Rainy | Winter |
|---|---|---|---|---|
| 1 | F.O.M. | 1 | 1.4 | 1 |
| 2 | L.O.D. | 4.55 | 4.20 | 10.2 |
| 3 | Total ash | 6.75 | 9.5 | 8.5 |
| 4 | water soluble ash | 1.70 | 2.50 | 3.50 |
| 5 | Acid Insolu. Ash | 1.25 | 1.70 | 1.0 |
| 6 | Sulphated Ash | 1.70 | 2.50 | 2.30 |
| 7 | Water S. Ext. V. | 10.10 | 10 | 32.8 |
| 8 | Alcohol S. Ext. V | 8.6 | 8.7 | 8.7 |
| 9 | Ether S. Ext. V | 4 | 4 | 3 |

**Table 4: Qualitative chemical tests J. adhatoda L leaf extract (+: Present, - : Absent)**

| S No. | Type of phytoconstituent | Season | | |
|---|---|---|---|---|
| | | Summer | Rainy | Winter |
| 1 | Alkaloids | + | + | + |
| 2 | amino- acids | - | - | - |
| 3 | Carbohydrates | + | + | + |
| 4 | Flavonoids | + | + | + |
| 5 | Glycosides | + | + | + |
| 6 | Phenolic compounds | + | + | + |
| 7 | Proteins | + | + | + |
| 8 | Steroids | + | + | + |
| 9 | Saponins | + | + | + |

## Qualitative chemical tests Presence of alkaloids Statistical analysis

**Table. 5 Monthly Variation of J. adhatoda with Altitude (% of alkaloids) (Morning) p< 0.05, n=3.**

| Altitude | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Low | 4.30 ±0.57 | 5.00 ±1.00 | 5.05 ±1.00 | 5.50 ±1.00 | 5.70 ±1.00 | 5.05 ±1.00 | 5.33 ±1.52 | 5.37 ±1.52 | 5.32 ±1.15 | 5.50 ±1.00 | 4.27 ±1.15 | 4.78 ±1.15 |
| Medium | 4.34 ±0.57 | 5.07 ±1.00 | 5.04 ±1.00 | 5.02 ±1.00 | 5.01 ±1.00 | 5.06 ±1.00 | 5.03 ±1.52 | 5.33 ±1.52 | 5.36 ±1.15 | 5.04 ±1.00 | 4.72 ±1.15 | 4.76 ±1.15 |
| High | 4.76 ±0.57 | 5.34 ±0.57 | 5.33 ±0.57 | 5.36 ±0.57 | 5.39 ±0.57 | 5.34 ±0.57 | 5.07 ±1.15 | 5.75 ±1.15 | 5.73 ±0.57 | 5.39 ±0.57 | 5.05 ±1.00 | 5.02 ±1.00 |



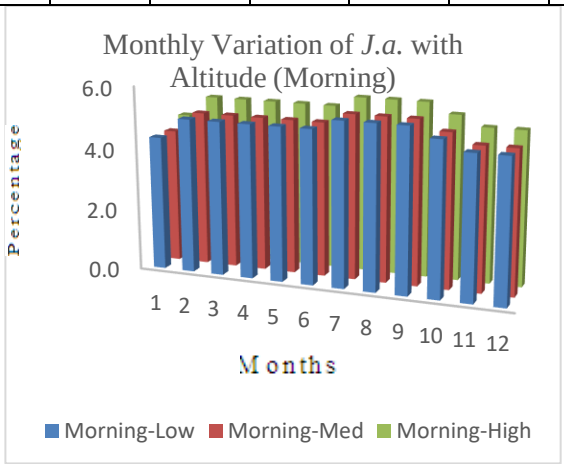



**Fig.- 1 Statistical plot- Variations with time and altitude**

The values are expressed as mean SEM; P<0.05, (Two way ANOVA followed by Tukey's multiple comparison test). The alkaloidal content in ethanolic extract of J. adhatoda is more and values are significantly variable in June- Sept. (rainy season) at morning time with high altitude place.

## HPTLC Statistical analysis

**Table 6 Monthly Variation of J. adhatoda with Altitude (Yield mg/g) (Morning) P< 0.05, n= 3.**

| Altitude | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Low | 1.00 ±0.20 | 1.10 ±0.26 | 1.25 ±0.55 | 1.43 ±0.11 | 1.82 ±0.05 | 2.10 ±0.05 | 2.27 ±0.05 | 2.34 ±0.05 | 2.23 ±0.11 | 1.42 ±0.05 | 1.25 ±0.10 | 1.62 ±0.60 |
| Medium | 1.624 ±0.20 | 1.56 ±0.32 | 1.84 ±0.65 | 2.03 ±0.10 | 2.46 ±0.11 | 2.78 ±0.05 | 2.84 ±0.05 | 2.93 ±0.05 | 2.82 ±0.15 | 1.97 ±0.10 | 1.72 ±0.10 | 2.15 ±0.60 |
| High | 2.03 ±0.58 | 1.84 ±0.05 | 2.17 ±0.60 | 2.44 ±0.11 | 2.83 ±0.05 | 3.17 ±0.05 | 3.22 ±0.05 | 3.36 ±0.05 | 3.24 ±0.11 | 2.49 ±0.05 | 2.23 ±0.10 | 2.32 ±0.05 |

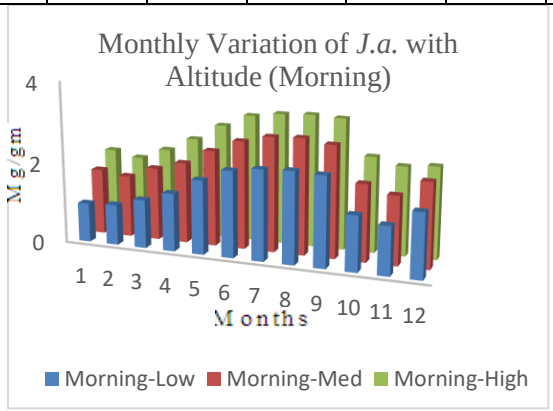





**Fig.- 2 Statistical plot- Variations with time and altitude**

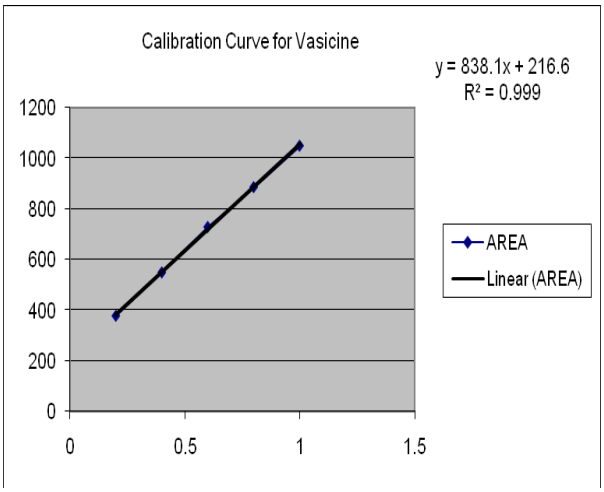
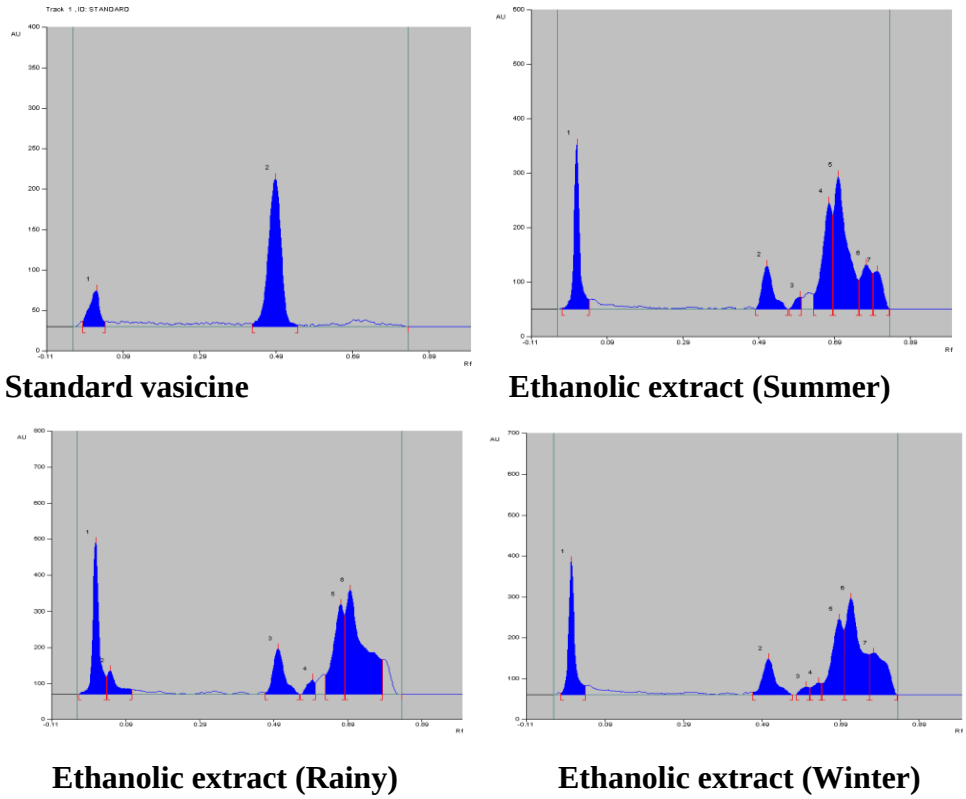



**Fig.- 3 Calibration curve of vasicine**

**Table 7: Concentration and A.U.C.**

| CONC µg/ml | AREA (AU) |
|---|---|
| 0.2 | 379.9 |
| 0.4 | 550.8 |
| 0.6 | 730 |
| 0.8 | 887 |
| 1 | 1049.9 |



**Standard vasicine** **Ethanolic extract (Summer)**

**Ethanolic extract (Rainy)** **Ethanolic extract (Winter)**

**Fig.- 4 Densitogram of vasicine**



**Table 8: HPTLC Analysis Of J. Adhatoda Leaf Extract**

| Rf Value | Season | Area (AU) | Yield (mg/g) |
|---|---|---|---|
| 0.49 | Summer | 1868 | 1.89 |
| | Rainy | 3149 | 3.20 |
| | Winter | 2329 | 2.36 |

The values are expressed as mean SEM; P<0.05, (Two way ANOVA followed by Tukey's multiple comparison test). The Vasicine content in ethanolic extract of J. adhatoda is more and values are significantly variable in June- Sept. (rainy season) at morning time with high altitude place.

## Discussion

The study of morphological, microscopical and physico-chemical parameters of Justicia adhatoda help to differentiate the plant from its other species. The pharmacognostic profile of plants presented here may be useful to supplement information with regard to its identification and will be helpful in establishing standardization criteria.

Present work is an attempt to compile data regarding variations of chemical constituents due to seasonal changes in selected plants i.e. Justicia adhatoda L. The plant belong to alkaloid category and possessing antidiabetic activity. The plant was authenticated by Botanical survey of India, Pune. Morphological and microscopic study was performed. The powdered drugs were subjected to phytochemical screening. Plant material in different seasons was extracted successively and as the percent yield of ethanolic extract found to be more as compare to other solvent extracts and according to solubility of selected phytoconstituents in ethanol, ethanolic extract was selected for further analysis. Qualitative chemical examination of extracts revealed presence of alkaloids, and other chemical components. Literature study proves that these constituents have antidiabetic activity.

The presence of vasicine in ethanolic extract of plant was confirmed by HPTLC fingerprinting and the content yield was calculated from AU. It was observed that, in different seasons there is a change in HPTLC pattern of the constituents i.e. in rainy season Vasicine content is more. It helps to identify best season for collection of plant material from the source so as to gain high yield of active component

and to increase the efficacy of the formulation.

## Conclusion

Seasonal variation is associated with the vegetative and reproductive stages of the plant, it has direct influence with the variation in chemical constituents of the plants. As per Ayurveda, there exists a huge collection of plants with antidiabetic potential. Only few of them have been scientifically proven and a lot more have yet to be explored and proved. Justicia adhatoda have shown varying degrees of HPTLC Chromatogram for vasicine and hence affects hypoglycemic potency in different seasons of collection. Future studies may target isolation, purification, and characterization of bioactive compounds present in these plants and formulation of a potent antidiabetic dosage form. The outcome of such studies may provide a starting point for selection of a particular season for collection of raw material to develop potential antidiabetic drugs.

Future studies may target isolation, purification, and characterization of bioactive compounds present in these plants and formulation of a potent antidiabetic dosage form. The outcome of such studies may provide a starting point for selection of a particular season for collection of raw material to develop potential antidiabetic drugs.

## Acknowledgments

Sincere gratitude should be expressed to Botanical Survey of India for authentication of plant samples. B.V.D.U.'s Poona College of Pharmacy for analytical study of extracts, JSPM's Jayawantrao Sawant College of Pharmacy and Research, Hadapsar, Pune for providing Laboratory facilities to perform the research work.

## Disclosure of conflict of interest

The authors declare that the research was conducted in absence of any conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial or not for profit sectors.

## List of abbreviations

A.U.C.- Area Under Curve
BSI- Botanical Survey of India
F.O.M.- Foreign Organic Matter
HPTLC- High Performance Thin Layer Chromatography
I.D.F.- International Diabetes Federation
L.O.D.- Loss on Drying OTC- Over the Counter
R & D- Research and Development
T. S.- Transverse Section
U.S- United States
WHO- World Health Organization

## References

Hiren BS, Manisha D, Sheju T., 2013. Compounds of selected Indian medicinal plants perspective on phytochemical and biochemical. International Journal of Chemical Research, 6 (2), 1- 4. DOI: 10.4172/2161-1009.1000323.

The International Union for Conservation of Nature (IUCN) Species Survival Commission Medicinal Plant Specialist Group (2011). DOI 10.2779/907382.

Marinelli J, ed. Plant: The Ultimate Visual Reference to Plants and Flowers of the World. New York, NY: DK. (2005). doi.org/10.1177/2156587212460241.

Gurib-Fakim A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 27, 1- 93. DOI: 10.1016/j.mam.2005.07.008.

Vyas BA., 2010. Phytopharmacological action of Pergularia daemia with special reference to its actions and mechanism of action as diuretic and anti-inflammatory agent. Ph. D thesis.

Adithan C., 1996. Pharmacology research in India, Indian Journal of Pharmacology, 28, 125- 128. DOI: 10.4155/tde-2016-0071.

Tandon V, Kapoor B, Gupta BM., 2004. Herbal drug research in India: A trend analysis using IJP as a marker, Indian Journal of Pharmacology, 36, 96-100. doi: 10.1155/2014/634723

Stumvoll M, Goldstein BJ, van Haeften TW., 2005. Type 2 diabetes: Principles of pathogenesis and therapy. Lancet. 365: 1333- 1346. DOI: 10.1016/S0140-6736(05)61032-X

American Diabetes Association. 2007. Standards of medical care in diabetes. Diabetes Care, 30, 4- 41. DOI: 10.2337/dc07-S004.

LK Keter, PC Mutiso., 2012. Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province, Kenya, 139 (1), 74- 80. DOI: 10.1016/j.jep.2011.10.014.

H Dong, N Wang, L Zhao, F Lu., 2012. Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis, 2012, Evidence-Based Complementary and Alternative Medicine, 1-12. DOI: 10.1155/2012/591654.

World Health Organization. Traditional medicine-growing needs and potential. 2002; 2, 1–6.

M Modak, P Dixit, J Londhe, S Ghaskadbi, TPA Devasagayam, 2007. Indian herbs and herbal drugs used for the treatment of diabetes, 40(3), 163- 173. doi: 10.3164/jcbn.40.163.

R Patil, B Ahirwar, D Ahirwar, 2011. Current status of Indian medicinal plants with antidiabetic potential: a review, 1(2), 291- 298. DOI:10.1016/S2221-1691(11) 60175-5.

PS Patil, R Shettigar, 2010. An advancement of analytical techniques in herbal research, 1(1), 8-14. doi.org/10.1155/2012/180103.

Mandal Bitasta, Madan Swati, 2011. Comparative standardisation and physicochemical evaluation of the leaves of *Stevia rebaudiana* Bertoni from different geographical sources. Pharmacognosy Journal, 3, 21- 26.