

A Comparative Study on Phytochemical And Biological Activities of two *Grewia* Species

Abstract

Background

Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as Nepal than to rest of the world. Inventorisation of herbal drugs used in traditional and modern medicines for a country like Nepal, appears to be a stupendous task, where a number of well established indigenous or traditional systems, including Ayurveda, Unani, Siddha, Homoeopathy, Tibetan, Amchi, Yoga and Naturopathy are practiced along with modern medicine for the management of total health care system.

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Method

Classic techniques for the solvent extraction of active constituents from medicinal plant matrices are based on the choice of solvent coupled with the use of heat. Soxhlet extraction is a general and well-established technique, which surpasses in performance other conventional extraction techniques. : In this method, finely ground sample of *Grewia* species were placed in a porous bag or "thimble" made from a strong filter paper or cellulose, which is placed, in thimble chamber of the Soxhlet apparatus. Extraction solvents were heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued. The resultant extracts were used for qualitative phytochemical analysis by color reactions, antibacterial effects by disc diffusion method and Human RBC membrane stabilization method was used to test the anti-inflammatory potential of the phytoconstituents.

Findings

Two species *Grewia asiatica* and *Grewia optiva* (family: Tiliaceae) from genus *Grewia* were studied for their phytochemical and biological properties. The result showed that the extractive value of *Grewia optiva* was found to be greater (2.56 % in hexane and 7.17 % in methanol) than *Grewia asiatica* (n-hexane 2.24% and methanol 5.04%) respectively. The test for the phytoconstituents for both species showed the presence of glycosides, steroids, tannins, flavonoids, terpenoids and saponins. No any extract of both species at dose of (0.25mg, 0.5mg and 1mg per cup) possessed a zone of inhibition against tested bacterial strains. The potency of n-hexane extract and methanol extract of both species for Human RBC membrane stabilization test were compared with standard Diclofenac potassium. HRBC stabilization and resulted a good membrane stabilization of n-hexane extract of *Grewia asiatica* (40.89%, 73.57%, 78.23%, 80.07%, 80.91% and 51.95%) at (50 µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml) than methanol extract of both species. The result of the both species possessed similar

phytochemicals and showed some membrane stabilization property which supports the traditional use.

Conclusion

Alkaloids were absent in the *Grewia* species extracts. The tested extracts have no any antibacterial effects against the tested bacterial stamps. The human RBC membrane stabilization tests were compared with standard Diclofenac potassium showed the good membrane stabilization of n-hexane extract of *Grewia asiatica* indicating the potent anti-inflammatory effect.

Key words: Human RBC membrane stabilization, n-hexane extract, *Grewia asiatica* and *Grewia optiva*,

INTRODUCTION

The different species of genus *Grewia* are commonly found in Nepal and used in traditional medicine. The two Species *Grewia subinaequalis* DC/ *asiatica* and *Grewia optiva* J.R.Drumm.ex Burret of family Tiliaceae found in local community have the possibilities of their use in modern medicine. [1]. 50% Ethanolic extract of aerial parts of *Grewia asiatica* showed hypotensive activity while the aqueous extract of stem bark is reported to be antidiabetic activity. Various species of genus *Grewia* contains the constituents such as flavonides, alkaloids, glycosides, tannins, steroids, triterpenoids etc. The major constituents are Quercetin, β -sitosterol [3], lupeol, stigmasteroletc [3,7]. The antibacterial test for *Grewia optiva* shows more zone of inhibition than doxycycline against

bacterial *Pseudomonas aeruginosa* [8]. The aerial part of *Grewia optiva* and results, antibacterial property having zone of inhibition of 11 mm against *Proteus merablus* (n-hexane extract) and 12 mm against *Bacillus cereus* (ethyl acetate extract) at concentration of 3mg/ml(100 μ l). No zone of inhibition was shown against *Staphylococcus aureus* and *E. coli*. But chloroform extract showed 9 mm of zone of inhibition against *E-coli* [9].Methanol and aqueous extract of *Grewia asiatica* root, bark results for showing analgesic and anti-inflammatory effect [10].Ethanolic extract of leaves of *Grewia asiatica* reduces in blood glucose level in on alloxan induced diabetic wister rats [11].

Materials and Methods

The fresh plant leaves were collected on July (rainy season) from Kavre district (876 m altitude) and identified as *Grewia optiva* and *Grewia asiatica* in National Herbarium and Plant Laboratory, Godawari, Lalitpur. The leaves were cut into pieces and were shade dried for a month at room temperature. Dried leaves were crushed into powder by electric blender and subjected to extraction by using continuous Soxhlet apparatus based on increasing polarity of solvent that is n-hexane and methanol. Each extract was tested for alkaloids, glycosides, tannins, saponins, reducing sugar, flavonoids, steroids and terpenoids using test procedure as below.

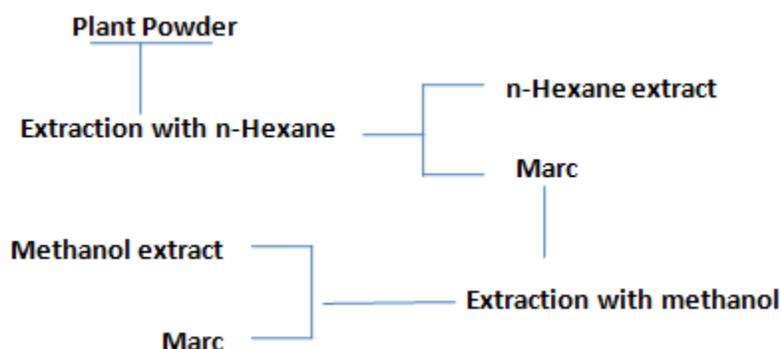


Fig 1: Extraction of plant powder in different solvents

Test procedure for phytochemical screening

Alkaloid: 6 ml solution was dissolved in 1.5 ml 2% HCl and then divided into two parts. To first part, 2-3 drops of Mayer's reagent was added and to second part add 2-3 drops of Bertrand's reagent (Silicotungstic Acid) was added separately. A white yellowish precipitate of first tube and white precipitate of second tube was observed indicating the presence of basic alkaloids. [9]

Tannin: A small quantity of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added to each of the filtrates. A dark blue or green solution was observed indicating the presence of tannins. [9]

Flavonoid: 4ml of extract was added to 1.5ml 50% methanol, few piece of metal magnesium also added and warmed. 5-6 drops of concentrated HCl is added. It was observed for the red color for the presence of flavonoid. [9]

Reducing compound: 0.5 ml extract was diluted by 1 ml water and add 0.5 ml Fehling solution A and Fehling solution B and warm. Red brick color was

observed to indicate the presence of reducing compounds.[9]

Saponin: 2ml of extract was placed in a test tube and shaken with water for 15 sec. Appearance of foam was observed to indicate the presence of saponin.[9]

Steroid: 4 mg extract was added with 0.5 ml acetic anhydride and 0.5 ml chloroform. Conc. H₂SO₄ was slowly added. Green bluish color was observed for the presence of steroid. [9].

Glycoside: Each extract was hydrolyzed with Hcl and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red precipitate was observed to indicate the presence of glycosides. [9]

Terpenoid: 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated H₂SO₄ was carefully added to form a layer. Formation of a reddish brown coloration at the interface was observed to indicate positive result. [9]

Antimicrobial test

Four pathogenic microorganisms were used for antibacterial test including two Gram positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29922) and two Gram negative (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). Doxycycline 30 mcg disc was used as reference standard for antibacterial assay.

Screening for antimicrobial activity

The antimicrobial test was determined by cup diffusion method and performed in two extract (n-hexane and methanol) of both plant species (*Grewia asiatica* and *Grewia optiva*) Stock solution of 40 mg/ml extract solution was prepared using 50% DMSO (methanol) and 1% tween 80 (n-hexane) and further diluted to 20 mg/ml and 10mg/ml using distilled water. The prepared and sterilized MHA was poured into sterile Petri plate of size 90 mm diameter, containing 20 ml of media. The plate containing hot media were allowed to solidification. Previously sub cultured bacterial suspensions were used by swab method using sterile cotton swab. Cups were made in MHA media plate with the help of sterile borer (5 mm) and labeled properly. To different cups 25µl of 40 mg/ml, 20 mg/ml and 10 mg/ml plant extracts were placed with the help of micropipette. Doxycycline 30 mcg disc was used as standard. The zone of inhibition was measured and experiment was conducted thrice.

Human RBC membrane stabilization method[12]

Blood sample and Reference drug

Blood sample was collected from healthy human volunteer who had not taken any Non steroid anti-inflammatory drugs for 2 weeks prior to this experiment and Diclofenac potassium was obtained from

Lomus pharmaceuticals Pvt. Ltd.

Preparation of Human Red Blood Cells (HRBC) Suspension.

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with normal saline (NS). The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Hypotonic Induced Hemolysis

The principle involved was stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contained 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypotonic sodium chloride solution [0.36 %], 0.5 ml HRBC suspension (10 % v/v) with 0.5 ml of plant extracts (50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml and 800µg /ml) and standard drug Diclofenac potassium of various concentrations (200µg/ml, 400µg/ml, 600µg/ml, 800 µg/ml) and control (distilled water instead of hypotonic sodium chloride solution to produce 100 % hemolysis) were incubated at 37°C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using UV spectrophotometer at 560 nm.

The % of HRBC membrane stabilization or protection was calculated as:

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

Percentage of protection:

$$= \frac{100 - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

RESULT AND DISCUSSION

The result for extractive value (percentage yield of crude extract) is shown in Fig 1. Similarly table 1 and table 2 represents the test results for phytochemicals and antimicrobial screening. The result of leaves extract of both plants showed that mass of polar component compound in greater proportion (Fig 1). Alkaloid is absent in all extract, that may be the possible reasons for not showing zone of inhibition in above antibacterial test. Alkaloids are the secondary metabolites in the plants, they usually toxic to various organisms and have drug like therapeutic agent. [13].

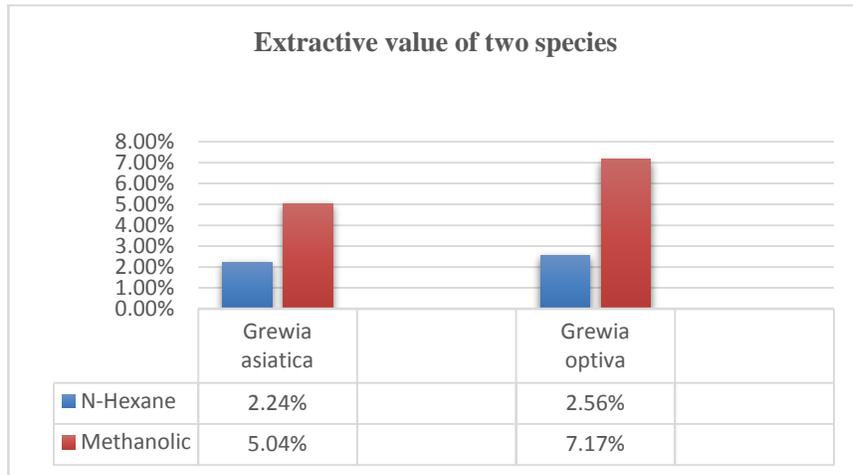


Fig 1: Extraction yield of both species in two solvents

Table 1: Phytochemical test of both species of two extract

Phytoconstituents	n-Hexane extract	Methanol extract
Alkaloids	absent	Absent
Glycoside	present	Absent
Terpenoids	Absent	Present
Steroids	Present	Present
Reducing sugar	Present	Absent
Flavonoids	Absent	Present
Saponins	Absent	Present
Tanins	Absent	Present

Table 2: Antimicrobial assay of two species in different concentration

Microorganisms	zone of inhibition of reference and both plant species	
Microorganisms	Doxycycline 30 mcg	Extracts 0.25 mg, 0.5 mg and 1 mg per cup
Staphylococcus aureus	17 mm	-
Escherichia coli	22 mm	-
Enterococcus faecalis	18 mm	-
Pseudomonas aeruginosa	21 mm	-

Note – no zone of inhibition

Membrane stabilization may be possible cause for anti-inflammatory action by inhibiting the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents include bactericidal enzyme proteinase, which up on extracellular release cause further tissue inflammation and damage [14]. The mode of action of extracts and standard anti-inflammatory drugs could be connected with binding to erythrocyte membranes with subsequent alteration of the surface

charges of the cells [15]. When RBC is subjected to hypotonic stress release of hemoglobin from RBC is prevented by anti-inflammatory agents because of membrane stabilization.

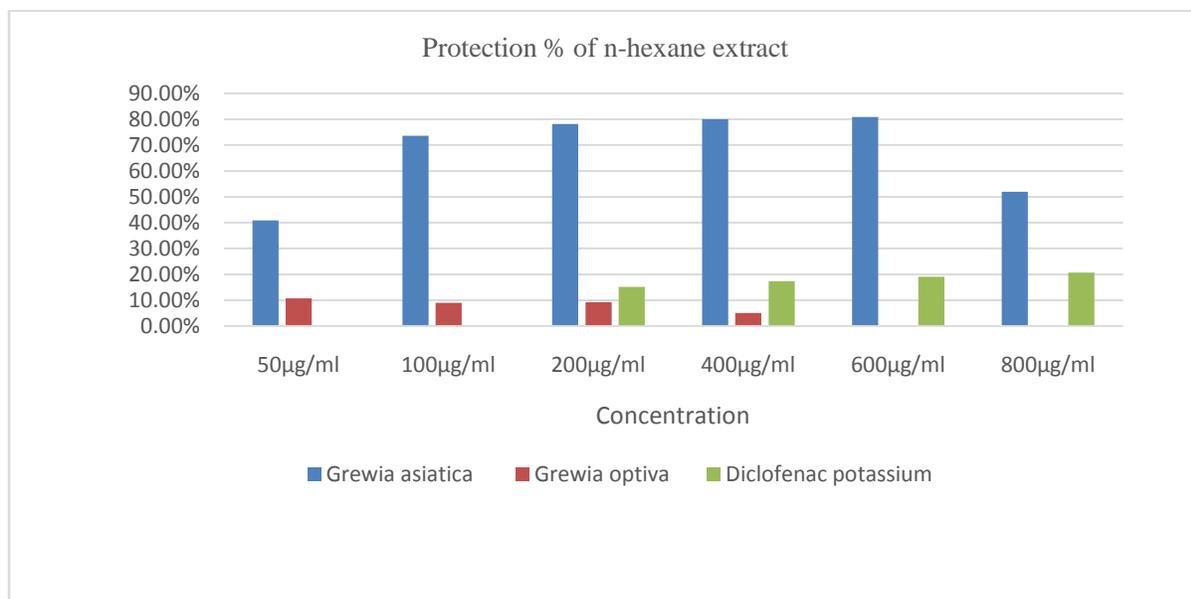


Fig 2: Percentage protection of n-hexane extract

So the stabilization of HRBC membrane by drugs against hypotonic sodium chloride solution induced hemolysis serves as a useful in vitro method for assessing the anti-inflammatory activity of various compound [16, 17]. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzyme and protease, which cause further tissue inflammation and damage up on extracellular release [18]. The result of RBC membrane is shown in fig 2 and fig 3.

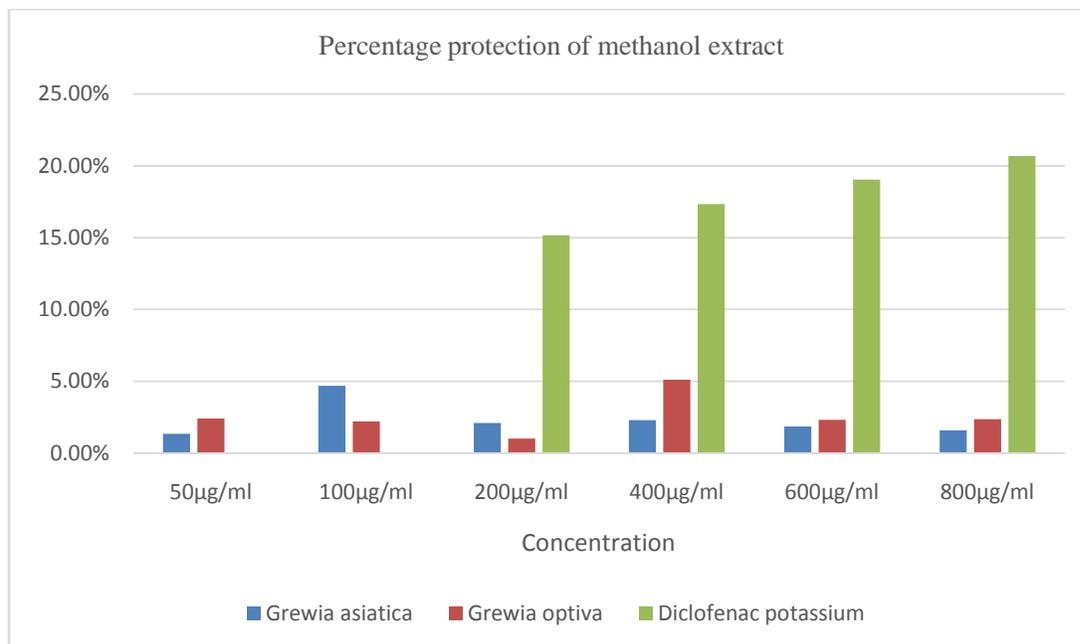


Fig 3: Percentage protection of methanol extract

n-hexane extract of *Grewia asiatica* showed the more percentage of protection than that of *Grewia optiva*. Percentage protection of n-hexane results 40.89%, 73.57%, 78.23%, 80.07%, 80.91% and 51.95% at 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, 600µg/ml and 800µg/ml respectively. The maximum percentage of protection was observed at 600µg/ml (80.91%). The inverse result was obtained in case of *Grewia optiva* at lower concentration they showed protection activity. The percentage protection showed 10.68%, 9%, 9.21%, and 5% at 50µg/ml, 100µg/ml, 200µg/ml, and 400µg/ml. The extract showed no percentage protection at 600µg/ml and 800µg/ml. The possible reason for showing protection activity on n-hexane extract may be due to presence of glycosides, reducing sugar and steroids. Plant steroids also showed anti-inflammatory activity [19]. However, methanol extract was positive for flavonoids, terpenoids, saponins, tannins, steroids, no good protection was observed. The highest percentage of protection was observed at 400µg/ml (5.12%). The possible reasons may be due to absent of those compounds (glycosides and reducing sugar) which can form protective interaction on cell membrane for stabilization on induced hypotonic stress.

CONCLUSION

The result of the membrane stabilization possesses modest anti-inflammatory activities which could be the rational for traditional claims and their uses could have some scientific basis. Thus it requires further investigation and researches on these plants to develop new drug candidate and their use in modern medicines.

REFERENCES:

1. Joshi, M., M. Patel, and P. Mehta, *Some folk medicines of Dangs, Gujarat state*. Bull Med Ethnobot Res, 1980. **1**.
2. Bhakuni, D., et al., *Screening of Indian plants for biological activity: Part III*. Indian Journal of Experimental Biology, 1971. **9**: p. 91-102.
3. Goyal, P.K., *Phytochemical and pharmacological properties of the genus Grewia: a review*. International journal of pharmacy and pharmaceutical sciences, 2012. **4**(4): p. 72-78.
4. Al-Youssef, H., M. Amina, and A. El-Shafae, *Biological evaluation of constituents from Grewia mollis*. Journal of Chemical and Pharmaceutical Research, 2012. **4**(1): p. 508.
5. Ullah, W., G. Uddin, and B.S. Siddiqui, *Ethnic uses, pharmacological and phytochemical profile of genus Grewia*. Journal of Asian natural products research, 2012. **14**(2): p. 186-195.
6. Sinha, J., et al., *Nutritional and medicinal potential of Grewia subinaequalis DC.(syn. G. asiatica.)(Phalsa)*. Journal of Medicinal Plants Research, 2015. **9**(19): p. 594-612.
7. Rehman, J., I.U. Khan, and M.N. Asghar, *Antioxidant activity and GC-MS analysis of Grewia optiva*. E3 J. Biotechnol. Pharma. Res, 2013. **4**(1).
8. Arora, S., *Antibacterial, antifungal, antioxidant and phytochemical study on the leaves extract of Grewia optiva*. Journal of Pharmacy Research, 2011. **4**(9).
9. Waliullah, G.U., et al., *Chemical Constituents and Biological Screening of Grewia optiva Drummond ex Burret Whole Plant*. American-Eurasian J Agric & Environ Sci, 2011. **11**(4): p. 542-546.
10. Paviaya, U.S., et al., *Analgesic and anti-inflammatory activity of root bark of Grewia asiatica Linn. in rodents*. Ancient science of life, 2013. **32**(3): p. 150.

11. Patil, P., M. Patel, and C. Bhavsar, *Preliminary phytochemical and hypoglycemic activity of leaves of Grewia asiatica L.* Res. J. Pharm. Biol. Chem. Sci, 2011. **2**: p. 516-520.
12. Chippada, S.C., et al., *In vitro anti inflammatory activity of methanolic extract of Centella asiatica by HRBC membrane stabilization.* Rasayan J Chem, 2011. **4**(2): p. 457-460.
13. Barbosa-Filho, J.M., et al., *Anti-inflammatory activity of alkaloids: A twenty-century review.* Revista Brasileira de Farmacognosia, 2006. **16**(1): p. 109-139.
14. Chou, C.T., *The Antiinflammatory Effect of an Extract of Tripterygium wilfordii Hook F on Adjuvant-induced Paw Oedema in Rats and Inflammatory Mediators Release.* Phytotherapy research, 1997. **11**(2): p. 152-154.
15. Hess, S. and R. Milong, *Inflammation Mechanism and Control.* Lepow. and Ward, PA ed. 1972, academic Press New York. p1-2.
16. Nambi, R., et al., *Satellite symposium on traditional medicine as Asian congress of Pharmacology.* Tamil University, Thanjavur, 1985. **140**.
17. Kumar, S., *Evaluation of RBC membrane stabilization and antioxidant activity of Bombax ceiba in an in vitro method.* Int J Pharma and Bio Sci, 2011. **2**(1).
18. Muruges, N., S. Vembar, and C. Damodaran, *Studies on erythrocyte membrane IV: in vitro haemolytic activity of oleander extract.* Toxicology letters, 1981. **8**(1): p. 33-38.
19. Das, S., et al., *Evaluation of anti-inflammatory activity of Clerodendron infortunatum Linn. extract in rats.* Global J Pharmacol, 2010. **4**(1): p. 48-50.