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Formulation and Evaluation of Herbal Gargle against Throat Irritation, Inflammation and Infection

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Abstract: Herbal gargles have gained significant attention as potential alternatives to conventional oral care products due to their perceived natural composition and reported therapeutic benefits. This review aims to provide a comprehensive overview of the efficacy of herbal gargles in promoting oral health and preventing various oral ailments. Our oral cavity is a sweet able place to grow different types of bacterial species either harmless or harmful for human. From ancient age medicinal plants are considered as a store room of different types of biological activity in Ayurveda, Unani and Siddha, and have important role to cleanse tooth and prevent different human pathogens are responsible for unpleasant odour, inflammation of teeth root, dental plaque. The study evaluates a wide range of herbal ingredients commonly found in mouth gargles, including essential oils, plant extracts, and traditional herbal remedies. It explores their antimicrobial properties, antioxidant activity, and potential for reducing plaque formation, gingivitis, and bad breath. Aqueous extract of Tulsi, Turmeric, Clove, Fennel, Betel leaves, Pudina, Ginger, Liquorice shows effective antimicrobial, antifungal activity, anti-inflammatory and anti-plaque properties. In this research work herbal gargle was evaluated depends on various parameter like color, pH, Phase separation, Homogeneity and antibacterial properties. Herbal gargles show potential as adjuncts to conventional oral care, Herbal gargle is suitable for any age group due to less side effect. Healthcare professionals and consumers should approach herbal gargles with caution, taking into consideration individual oral health needs and consulting oral healthcare providers for personalized recommendations.

Keywords: Herbal Gargle, Formulation, Evaluation, Antimicrobial study, E. Coli

I. INTRODUCTION

The first known references to mouth rinsing are described in Ayurveda and Chinese medicine in 2700 BC. Mouth gargle is a chemotherapeutic agent used as effective home care system by the patient. [1]

In the Greek and Roman periods, mouth rinsing became common among the Hippocrates. They recommended a mixture of salt, alum and vinegar.[1]

Gargles are aqueous solutions used to treat the problem related to pharynx and nasopharynx by pushing air from the lungs through the gargle while it is held in the throat. Often, gargles need to be diluted with water before use. Gargles are used to get the medication onto the mucosal surface of the throat.[2,3] The preparations need to have acceptable organoleptic qualities and be quick-acting.

1) *Natural:* Natural gargles which are also known as herbal gargles. eg: liquorice, clove, ginger, salt water.

2) *Chemical:* Gargles, made from chemical compound. eg.: Methyl salicylate, Saccharine sodium.

Such a variety of health advantages come from gargling and rinsing with salt water. It promotes good oral hygiene and dental health with gargling and it supports the postoperative care process. It aids in the recovery of canker sores. Sometimes pregnancy difficulties may be avoided with its aid.





Gargle can be dangerous for children when used orally they didn't even know how to gargling. Due to oral cavity sometimes, gargle might produce sensation and itching in mouth. [4]






II. MATERIALS AND METHODS



A. Collection of Plants

Leaves, stem, buds and dried seeds of Tulsi (*Ocimum sanctum*) [5,6], Clove (*Eugenia caryophyllus*) [7,8], Fennel (*Foeniculum vulgare*) [9,10], Ginger (*Zingiber officinale*) [11,12], Liquorice (*Glycyrrhiza glabra*) [13,14], Turmeric (*Curcuma longa*) [15,16], Fenugreek (*Trigonella foenum-graceum*) [17,18], Cardamom (*Elettaria cardamomum*) [19,20], Betel Leaves (*Piper betel*) [21,22], Pudina (*Mentha piperita* Linn) [23,24], Coriander (*Coriandrum sativum*) [25,26] were randomly collected from mature plants.

TABLE-1 List Of Ingredients With Their Functions

| Sl.No | Ingredients | Scientific name | Chemical constituents | Plant part | Functions |
|-------|--|-----------------------------|--|--|---|
| 1 | Tulsi family: <i>Labiatae</i> plant part: fresh leaves | <i>Ocimum sanctum</i> | Eugenol, Estragole, Camphene |  | Antibacterial, insecticidal and stimulant |
| 2 | Clove family: <i>Myrtaceae</i> Plant part: dried flower buds | <i>Eugenia caryophyllus</i> | Eugenol, Caryophyllene, Methyl eugenol |  | Dental analgesic, carminative, stimulant, antioxidant, flavouring agent, an aromatic and antiseptic |
| 3 | Fennel family: <i>Umbelliferae</i> Plant part: dried ripe fruit of plant | <i>Foeniculum vulgare</i> | Fenchone, Anethole, Estragole |  | Carminative, aromatic, stimulant and flavouring agent |
| 4 | Ginger family: <i>Zingiberaceae</i> Plant part: rhizomes | <i>Zingiber officinale</i> | Gingerol, Shogaol, Zingiberene |  | Stomachic, an aromatic, a carminative, stimulant and flavouring agent. |


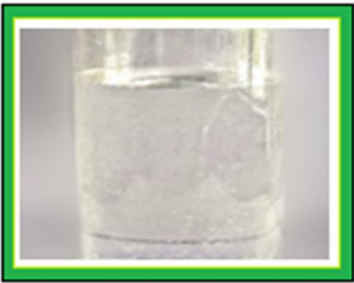
| | | | | | |
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| 5 | <p>Liquorice: family: Leguminosae Plant part: dried peeled or unpeeled roots and stolons of the plant</p> | <i>Glycyrrhiza glabra</i> | Glycyrrhizin, Chalcone, Enoxolone |  | Demulcent, mild expectorant. sweetening agent, flavouring agent, antitussive, antispasmodic, anti- inflammatory and antiulcer drug |
| 6 | <p>Turmeric: family: Zingiberaceae Plant part: dried, as well as fresh rhizomes of plant</p> | <i>Curcuma longa</i> | Curcumin, Phellandrene, Curcuminoid |  | Expectorant, a condiment or spice, colouring agent |
| 7 | <p>Fenugreek Family: Fabaceae Plant part: Fresh and dried seeds</p> | <i>Trigonella foenum- graceum</i> | Trigonelline, Diogenin, Sotolone |  | Antioxidant, antiulcer, antibacterial, neuroprotective and liver protection propaties |
| 8 | <p>Cardamom: Family: Zingiberaceae Plant Part: dried ripe fruits</p> | <i>Elettaria cardamomu m</i> | Terpineol, Eucalyptol, Sabinene |  | Aromatic, carminative, stimulant and flavouring agent |
| 9 | <p>Betel leaves Family: Piperaceae Plant part: Fresh leaves</p> | <i>Piper betel</i> | Arecoline, Methyl euginol, Estragole |  | Pungent and flavouring agent |

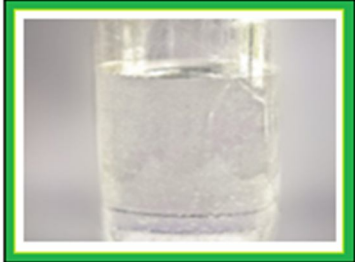
| | | | | | |
|----|---|-------------------------------|-------------------------------------|--|--|
| 10 | Coriander Family: Umelliferae Plant part: fully dried ripe fruits of the plant | <i>Coriandrum sativum</i> | Dodecanal, p- Cymene, Borneol |  | Aromatic, carminative, stimulant and flavouring agent |
| 11 | Pudina Family: Labiatae Plant part: Fresh leaves | <i>Mentha piperita</i> | Menthol, Menthone, Eucalyptol |  | Carminative, stimulant, aromatic, counter- irritant flavouring agent and mild antiseptic |

B. Collection of Chemicals

In this formulation Sodium Benzoate is used as preservative, Sorbitol is used for soothing & refreshing and Glycerol is used to treat mouth irritation.

TABLE-2 Chemicals Used In The Preparation Of Gargle

| Sl. No. | Ingredients | Raw materials | Functions |
|---------|-----------------|--|---|
| 1 | Sodium Benzoate |  | Anti-corrosive and preservative |
| 2 | Sorbitol |  | Provide a soothing feeling in mouth , mild sweetness and a refreshing. Resistance to dental caries. |

| | | | |
|---|----------|--|--|
| 3 | Glycerol |  | Treating sore throat pain, mouth pain and minor mouth irritation |
|---|----------|--|--|

C. Aqueous Extraction Process of Plant parts-

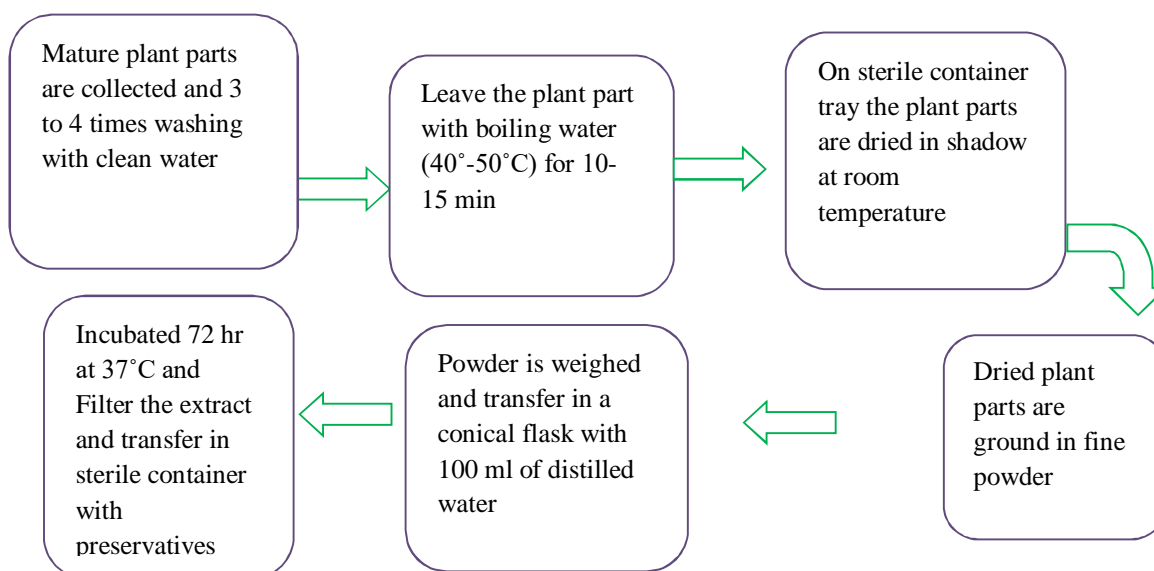


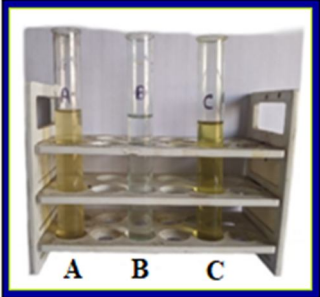
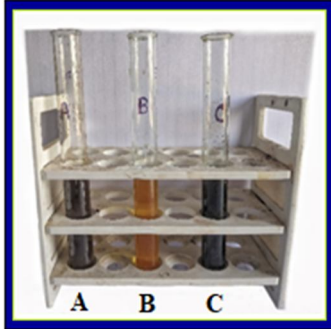

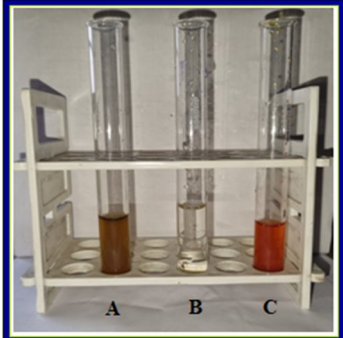
TABLE-3 : Water Soluble Extracts

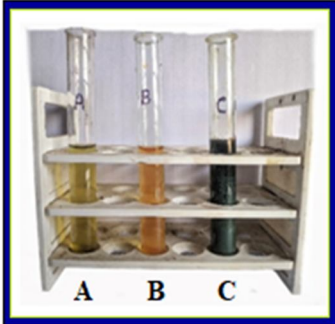
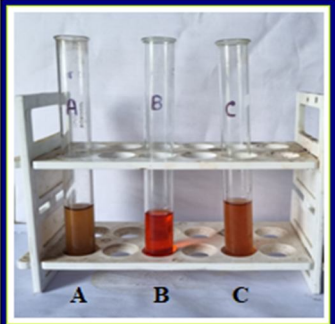
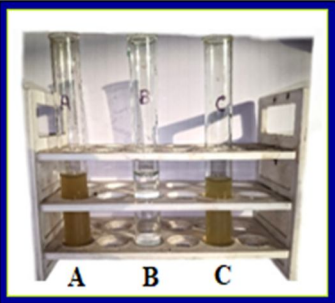

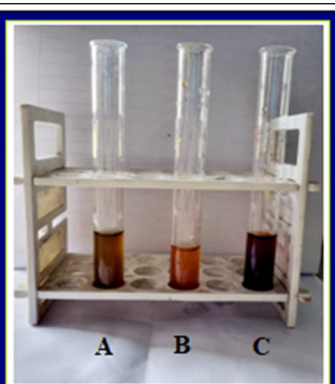
| Ingredients | Amount taken in gm | Water quantity (ml) | Extract (gm) | Percentage (%) |
|--------------|--------------------|---------------------|--------------|----------------|
| Tulsi | 1.02 | 50 | 0.144 | 14.4 |
| Clove | 1.01 | 50 | 0.2 | 20 |
| Fennel | 1.02 | 50 | 0.219 | 21.9 |
| Turmeric | 1.03 | 50 | 0.267 | 26.7 |
| Ginger | 1.01 | 50 | 0.224 | 22.4 |
| Liquorice | 1.02 | 50 | 0.28 | 28 |
| Coriander | 1.03 | 50 | 0.209 | 20.9 |
| Cardamom | 1.01 | 50 | 0.216 | 21.6 |
| Betel leaves | 1.03 | 50 | 0.186 | 18.6 |
| Fenugreek | 1.04 | 50 | 0.236 | 23.6 |
| Mint | 1.02 | 50 | 0.193 | 19.3 |


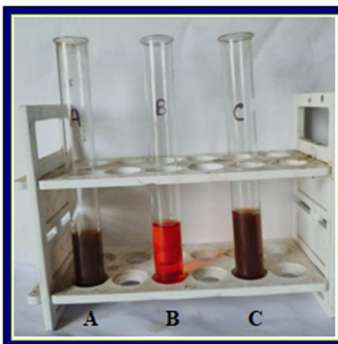
D. Phytochemical Screening

The chemical, identification tests of different herbal extract are given in the following

TABLE-4 Chemical Test Of Plant Extracts

| Sr. No. | Name of herbs | Tests | Observation | Images |
|---------|---------------|--|--|---|
| 1 | Tulsi | 0.5 ml of crude extract was mixed with 2ml of glacial acetic acid containing 2-3 drops of 2% solution of FeCl_3 . Then 2ml of concentrated H_2SO_4 was poured into the mixture. | A brown ring at the interface indicated the presence of cardiac glycosides |  |
| 2 | Clove | Aq. Extract + ferric chloride 5% solution | the cloves extract and eugenol standard turned light green |  |
| 3 | Turmeric | 1 or 2 ml of extract added with Wagner's reagent (dissolving 2gm of iodine and 6 gm of potassium iodine in 100ml of distilled water) | Reddish brown precipitate shows for the alkaloids |  |
| 4 | Liquorice | Moistened with 80% H_2SO_4 | Orange yellow is observed for transformation of flavone glycoside Liquiritin to chalcone glycoside isoliquiritin |  |

| | | | | |
|---|--------------|---|---|---|
| 5 | Pudina | 1ml of Aqueous extract and 2 drops of Ferric chloride(FeCl_3) added with the extract | Green – blackish colour indicate presence of catechol tannins |  |
| 6 | Cardamom | Aqueous extract of Elettaria cardamomum + volatile oil + Sudan III | Red colour due to volatile oil |  |
| 7 | Fennel | 2ml extract + 1ml Mayer's reagent (1.36gm mercuric chloride + 5gm potassium iodide in 100ml water) | Light green precipitate indicating presence of alkaloids |  |
| 8 | Ginger | Aq. Extract of ginger + few drops of Wagner's reagent (dissolving 2gm of iodine and 6 gm of potassium iodine in 100ml of distilled water) | Reddish brown precipitation due to alkaloids |  |
| 9 | Betel leaves | 1 gm Powder form of Piper betel + 10 ml distilled water (5 min boil) + 2 drop 5% FeCl_3 | Greenish black Precipitation due to tannin |  |

| | | | | |
|----|-----------|---|---|--|
| 10 | Fenugreek | 4ml of aqueous extract + few drops of Wagner's reagent | Green precipitate confirms the present of alkaloids |  |
| 11 | Coriander | Aq. Extract of <i>Coriandrum sativum</i> + Violet oil + Sudan III | Red colour due to volatile oil |  |

Here, A= Plant Extract, B= Reagent, C= Final Product

E. Formulation of herbal gargle

The herbal Mouth-gargle was prepared by the formula given in Table – 5

TABLE-5 Formulation Table

| Ingredients | Function | Each 5 ml contains |
|-------------|------------------|--------------------|
| Tulsi | Antibacterial | 20% |
| Ginger | Stomachic | 5% |
| Clove | Dental analgesic | 10% |
| Liquorice | Sweetening agent | 5% |

| | | |
|-----------------|------------------|-------|
| Cardamom | Aromatic | 3% |
| Turmeric | Expectorant | 5% |
| Fenugreek | Antioxidant | 5% |
| Fennel | Carminative | 10% |
| Betel leaves | Pungent | 3% |
| Pudina | Cooling agent | 10% |
| Coriander | Carminative | 3% |
| Sorbitol | Sweetening agent | 10% |
| Glycerol | Co surfactant | 5% |
| Sodium benzoate | Preservatives | 1.25% |
| Water | Diluent | q.s |

III. EVALUATION

- 1) *Colour*- Dark Brownish
- 2) *Odour*- Characteristics
- 3) *pH*- pH of prepared herbal gargle was measured by using Digital pH meter. The pH meter was previously calibrated using standard buffer solution. Collect about 1 ml of gargle and dissolved in 50 ml of distilled water and it's pH was found 5.84 at room temperature.
- 4) *Weight per ml*:

Table – 6: Weight per ml test

| Sample quantity | Weight of empty volumetric flask | Weight of volumetric flask with formulation | Result (wt/ml) |
|-----------------|----------------------------------|---|----------------|
| 25ml | 42.21 gm | 67.53 gm | 1.01gm/ml |

5) *Test for Microbiological growth*

The formulated mouth gargle was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were kept at 37°C for 24 hours in the incubator. After the incubation period plates were taken out and checked for microbial growth.

6) Stability studies

Stability test aims to ensure that the gargle formulations are usable and can maintain the same characteristics in the long term basis, before undergoing antibacterial assay

The formulation and preparation of a pharmaceutical product is incomplete without proper stability studies of the prepared product. This is done in order to determine the physical and chemical stability of the prepared product and thus determine the safety of the product at ($30 \pm 2^\circ\text{C}$, RH- $65 \pm 5^\circ\text{C}$)

TABLE-7 : Stability Studies Of Formulated Herbal Gargle

| Sl. No. | Parameters | Observations | | | |
|---------|------------------|-----------------|-----------------|-----------------|-----------------|
| | | Initial | 10 days | 20 days | 30 days |
| 1 | Colour | Dark brown | Dark brown | Dark brown | Dark brown |
| 2 | Odour | Characteristics | Characteristics | Characteristics | Characteristics |
| 3 | Consistency | Stable | Stable | Stable | Stable |
| 4 | Phase separation | Nil | Nil | Nil | Nil |
| 5 | pH | 5.83 | 5.82 | 5.84 | 5.85 |
| 6 | Homogeneity | Good | Good | Good | Good |
| 7 | Weight /ml | 1.01 gm/ml | 1.03 gm/ml | 1.01 gm/ml | 1.02 gm/ml |

IV. RESULT & DISCUSSION

The look of gargle retained its colour and homogeneity after a one-month examination. The gargle's formulation showed no signs of phase separation. Gargle has maintained its pH and has a mildly acidic character. The specially prepared gargle proved active against a variety of human pathogens, including *S. aureus* (+ve) and *E. coli* (-ve).

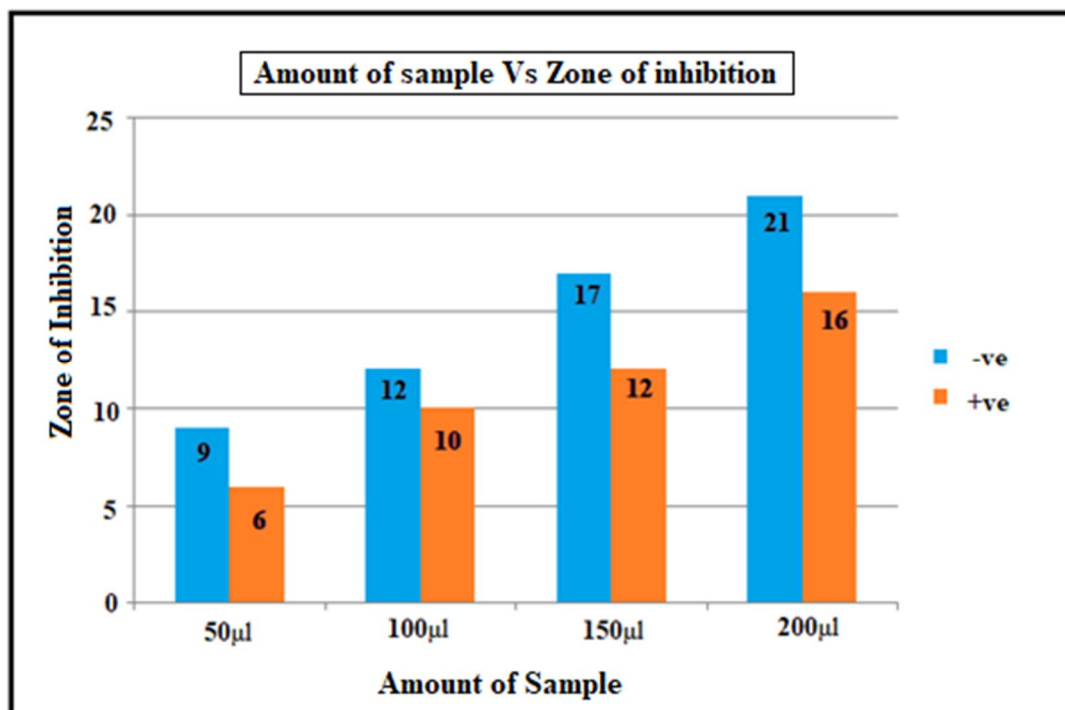
The formulation was useful against various microorganisms and shows good stability during various physicochemical test and mostly effective against gram negative bacteria instead of gram positive bacteria. This mouth-gargle entirely prepared from plants parts and safe for health.

TABLE-8 : Zone Of Inhibition

| ORGANISM | ZONE OF INHIBITION (nm) | | | |
|-----------------|-------------------------|-------------|-------------|-------------|
| | 50 μ l | 100 μ l | 150 μ l | 200 μ l |
| E. coli(-ve) | 9 nm | 12 nm | 17 nm | 21 nm |
| S. aureus(+ve). | 6 nm | 10 nm | 12 nm | 16 nm |

For various gargle concentrations amount, the agar diffusion method was used to determine their antibacterial activity. For E. coli (-ve), the zone of inhibition was found to be 9 mm for 50 μ l, 12 mm for 100 μ l, 17 mm for 150 μ l, and 21 mm for 200 μ l, respectively. For S. aureus, the zone of inhibition was found to be 6 mm for 50 μ l, 10 mm for 100 μ l, 12 mm for 150 μ l, and 16 mm for 200 μ l, respectively. These findings demonstrated the significant antibacterial activity of the herbal mouthwash and the capability of the current product to prevent bacterial growth in the oral cavity.

TABLE- 9 ANTI-BACTERIAL ACTIVITY



V. CONCLUSION

The present liquid herbal gargle can work in long way to help people to cure the various throat disorder. Present herbal formulation is acceptable for a long period.

Furthermore the prepared herbal gargle were standardized by various physicochemical studies like pH, appearance of solution, consistency, phase separation and microbial study and all test results are in limit. So the prepared herbal formulation is very good and safe for any age group.

VI. ACKNOWLEDGEMENT

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VII. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to reveal.

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