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FORMULATION AND EVALUATION OF POLYHERBAL ANTIFUNGAL MEDICATED SOAP FOR SKIN DISEASES

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ABSTRACT

Piper *betel* and Aloe *vera* is a popular medicinal plant in Asia. These Plants have been used as a traditional medicine to treat various dermatological conditions. It is highly abundant and inexpensive, therefore promoting further research and industrialization development, including in the food and pharmaceutical industries. This current review showed that betel leaves and stem extract, Aloe *vera* juice soap could inhibit fungal growth and kill various fungal species. The zone of inhibition indicated fungicidal and fungistatic effects. The present study highlights the formulation and evaluation of polyherbal soap using the aqueous extract of Piper betel leaf and stem powder and Aloe *vera* juice. The constituents of the plants have been demonstrated to exhibit antifungal, antibacterial, anti-inflammatory, antioxidant properties. Herbal soap ingredients Aloe *vera* juice, piper betel, glycerin, coconut oil, sodium hydroxide were used, and different formulations of soap were prepared. The evaluation of antifungal activity of F1, F2, F3, F4 were carried out by disc diffusion method. From the evaluation studies the soap containing 1g betel extract and 1g Aloe *vera* juice (F4) were found to be most active. Aloe *vera* gel increases the foam retention of the formulated soap.

KEYWORDS: Anti-fungal, Piper *betel*, Aloe *vera*, Aq. Extract, Medicated Soap.

INTRODUCTION

Herbal therapy has increased in popularity in the past two decades among patients seeking alternative treatments to conventional western allopathic medicine. The number of visits to alternative medicine practitioners in the United States has grown rapidly and in 1997 it was estimated to be 629 million, surpassing the number of visits to all primary care physicians. With alternative herbal therapies, an individual patient often treats himself or herself, many times without high-quality professional advice. Patients are advised to ensure the safe use of herbal therapies by deciding on health goals; informing themselves on efficacy, safety, interactions, and usage of the medicine; selecting therapies that are likely to achieve their goals; having a correct diagnosis before using the therapy; consulting reputable practitioners; informing the practitioners about all the remedies they are using; monitoring the effects of the remedies, both positive and negative; waiting patiently for effects to become noticeable; and adjusting doses as needed to accommodate surgery, illness, or changes in conventional .Class 1 herbs being safe to consume appropriately, Class 2 safe to consume with restrictions (2a for external use only, 2b not for use in pregnancy, 2c not for use while nursing, and 2d indicating other specific restrictions), Class 3 herbs restricted to use only when supervised by an expert, and Class 4 herbs have insufficient data for classification of safety.[1]

Medicinal plants have many characteristics when used as a treatment, as follow:

• Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

• Preventive medicine- It has been proven that the component of the plants also characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.[2]

Skin Infections

An infection of the skin that can be caused by bacteria, fungus, viruses or parasites. Skin disease may cause:

- Discolored skin patches (abnormal pigmentation)
- Open sores, lesions or ulcers.
- Peeling skin

- Rashes, possibly with itchiness or pain.
- Red, white or pus-filled bumps.
- Scaly or rough skin.[3]

Bacterial skin infections often begin as small, red bumps that slowly increase in size. Some bacterial infections are mild and easily treated with topical antibiotics, but other infections require an oral antibiotic. Different types of bacterial skin infections include: Impetigo, boils, leprosy.

Viral skin infections are caused by a virus. These infections range from mild to severe. Different types of viral infections include: Chickenpox, Warts and Hand foot and mouth disease.[4]

Fungal infections: Fungi exist in two basic forms: Yeasts and molds. Yeasts are typically single, small, and oval cells, whereas mold colonies consist of filamentous strands called hyphae. Invasive fungal infections are characterized by high morbidity and mortality, although these infections are now more frequent they are still difficult to diagnose, prevent, and treat.[5] A fungal infection, also called mycosis, is a skin disease caused by a fungus. There are millions of species of fungi. They live in the dirt, on plants, on household surfaces, and on your skin. Sometimes, they can lead to skin problems like rashes or bumps.

Fungal Infection Symptoms

- Irritation
- Scaly skin
- Redness
- Itching
- Swelling

Treatment

Creams, lotions, or sprays, readily penetrate into the stratum corneum to kill the fungi (fungicdal agents), or at least render them unable to grow or divide (fungistatic agents). Miconazole, clotrimazole, and ketoconazole are fungistatic.Allylamines and benzylamines such as terbinafine, naftifine, and butenafine are fungicidal, actually killing the fungal organisms.[6]

Side Effects

Oral antifungal drugs are more toxic to the human body as compared to topical antifungal drugs. They show various side effects on the application site, such as burning, redness, and some allergic reaction.[7]

Mechanism of Action of Antifungal Drugs

Antifungal drugs attacks the structure and function of the fungal cell. They bind irreversibly to cell membranes. Alteration of the permeability of these structures precedes metabolic disruption and cell death. Griseofulvin deteriorates spindle and cytoplasmic microtubules, influencing cell division and outgrowth of hyphal tips. Flucytosine is deaminated to 5-fluorouracil, which is then phosphorylated and incorporated into RNA; protein synthesis is consequently impaired. A mechanism of action via inhibition of DNA synthesis is an alternative explanation. The imidazole derivatives inhibit the biosynthesis of ergosterol, the main sterol in membranes of fungi.[8]

Role of Herbal Antifungal Drugs

Antifungal drugs have undesirable side effects or are very toxic, induce drug-drug interactions, or lead to the development of resistance. Some drugs are also ineffective and have become less successful as therapeutic agents. Thus, searching for alternative antifungal drugs has been a major concern in recent years. Natural products play an important role in drug development programs in the pharmaceutical industry. As a result, several medicinal plants have been extensively studied in order to find safe, less toxic, and more effective drugs. Some of the antifungal drugs most recently introduced in clinical practice are echinocandines and sordarines derived from natural products. Herbal formulations always have attracted considerable attention due to their good activity and comparatively lesser side effects when compared to synthetic drugs.[9]

Soap

A soap is a salt of a compound, known as a fatty acid. A soap molecule has a long hydrocarbon chain with a carboxylic acid group on one end, which has ionic bond with metal ion, usually sodium or potassium. The hydrocarbon end is non polar which is highly soluble in non-polar substances and the ionic end is soluble in water [10]. They are carboxylate salts with very long hydro carbon chains. They can be made from the base hydrolysis of a fat or oil. It is used as a surfactants for washing, bathing and cleaning but used in textile spinning

for lubricants saponification is the process in making the soap by reaction of triglyceride fats are hydrolyzed into free fatty acids then it will combine with alkali to forming crude soap.[11]

The cleaning action of soaps are because of their ability to emulsify or disperse water-insoluble materials and hold them in the suspension of water. This ability is seen from the molecular structure of soaps. When soap is added to water that contains oil or other water-insoluble materials, the soap or detergent molecules surround the oil droplets. The oil is, dissolved in the alkyl groups of the soap molecules while the ionic end allows it to be dissolved in water. As a result, the oil droplets are to be dispersed throughout the water and can be washed away.[12]

MATERIALS AND METHODS

Collection Identification and Processing of Plant

The leaves and stem of *Piper betle* and leaves of *Aloe barbadensis* were collected from Kasaragod district in the month of December. The plant material was taxonomically identified by the Botanist. *Piper betle* were dried under shade for about 15 days and then powdered with a mechanical grinder and stored in an air-tight container.

Aloe vera gel was collected by Cut a mature leaf from the plant, Slice the leaf in half lengthwise. Cut along the aloe leaf's length to reveal the clear gel inside. Squeeze the gel out, and store.[13]

EXTRACTION OF LEAVES AND STEM

Extraction of dried powder of the stem, leaves of *piper betle* was carried out by Maceration technique. Extraction is done using chloroform water. Around 20g of dried powder was weighed and dissolved in 180ml of chloroform water. Maceration was carried on a heavy rotary shaker (4 days for leaves and 7 days for stem). The extract was filtered and solvent distilled off and finally the dried extract was obtained.[14]

PREFORMULATION STUDY

Preformulation studies may be defined as testing of the physical and chemical properties of a drug substance alone and in combination with excipients proposed to be used in formulation. Preformulation investigations are designed to deliver all necessary data, especially physico-chemical, physico-mechanical and biopharmaceutical properties of drug substances, excipient and packaging materials as well as compatibility. The overall objective of the preformulation

testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be produced.[15]

INCOMPATIBILITY STUDY BY FTIR

The compatibility between drug and excipients was carried out in the ratio of 1:1. API and excipients are thoroughly mixed in pre-determined ratio of 1:1 and passed through sieve no 40. The blend was filled in transparent glass vials and were closed with grey colored rubber stoppers and further sealed with aluminium seal and charged into stress condition. Similarly, API should also be kept at all condition as for the samples. Samples were withdrawn for analysis within two days of sampling date as per the compatibility study plan. Physical observation should be done at every week up to one month and FTIR study was carried out to determine the compatibility of excipients with the drug.

FORMULATION OF SOAP

Basic glycerin soap was formulated by using 10g of coconut oil, 1.7g of sodium hydroxide, 3.3ml of water and 1ml of glycerine. Weighed 1g, 0.5 g of aqueous extract of piper betel and aloe vera gel. Glycerin basic soap was melted and the above Extract was incorporated into melted solution with continuous agitation for 30 minutes until molten mixture became homogeneous. The semisolid mixture was poured into a mould and allowed to solidify.[16]

PROCEDURE FOR SOAP PREPARATION

COLD PROCESS: When using thermometer in this experiment never use it to stir liquids. Instead, use a fire polished glass rod. To properly measure the temperature of a liquid, whole the thermometer so that its bulb is suspended in the center of the liquid while reading the mercury level.

Weigh 150 ml beaker on the trip scale and add NaOH pellets to it. Handle NaOH with care as NaOH can burn the skin and is especially harmful to the eyes. Add cold water to the beaker. Stir the mixture of NaOH pellets and water, until a clear solution results. Weigh 250ml beaker on the trip scale and add fat to it. Then place the beaker on the hot plate with low heat and with occasional stirring. Warm the fat to between 42-50˚C remove the fat from hot plate and add the Lye solution to the fat with stirring. Add perfume or other additives at this point. Pour the emulsion into a plastic cup and place it for the reaction to run. Keep it for 2weeks. During this time a powdery layer of sodium carbonate will form on the surface, as residual

NaOH reacts with CO2 in the air while the soap is drying. This powdery layer should be sliced off and the soap is ready to be used.[17]

SOAP BASE FORMULATION

Cold process method: For the preparing soap base, we take 10g of coconut oil in a 100ml of beaker. Put it on the water bath boil the liquid up to forming strong consistency under the temperature 40-45℃ with stirring. NaOH was weighed into a clean beaker and add into the distilled water. Add this solution to the coconut mixture, boil at 40-45℃ up to formation of base consistency. Then the mixture can be transfer into soap molds allow 2hr then soap will be formed. (TABLE 1)

POLY HERBAL SOAP FORMULATION

To prepare soap we take the required quantity of soap base in a 500ml of beaker and maintain the temperature at 45℃ to heat the soap base on the water bath without stirring. Then the soap base will be converts into liquid form. Glycerin, *Piper betel* extract, aloe vera juice, added to the above mixture. Boil the mixture 45℃ on the water bath to obtain proper mixture without stirring. Then the mixture poured into the soap molds, after 2-3 hours remove the soap from molds.[18] Uses of ingredients used in the herbal soap formulation.(TABLE 2)

FORMULATION DEVELOPMENT

Different formulations (F1, F2, F3, and F4) of soap were prepared by changing the concentrations of active constituents *(piper betle*, aloe vera juice). (TABLE 3)

EVALUATION

Physical Appearance

The prepared gel formulations were inspected visually for their color, odour and appearance.

pH

The pH of all the formulations was determined by using digital pH meter. The formulations were dissolved in 100 ml of distilled water and stored for 2 hours. The measurement of pH of formulation was done in previously calibrated pH meter.[19]

FOAM RETENTION

25 ml of the one percent soap solution was taken into a 100 ml measuring cylinder. The cylinder was covered with hand shaken 10 times. Volume of foam at 1 minutes interval for 4 minutes was recorded.[20]

FOAM HEIGHT

0.5 grams of sample of soap was taken dispersed in 25 ml distilled water. Then, transferred it in to 100ml measuring cylinder; volume was make up to 50 ml with water. 25 strokes were given and stand till aqueous volume measured up to 50 ml and measured the foam height, above the aqueous volume.[21]

ALCOHOL INSOLUBLE MATTER

5gm of sample was taken in a conical flask. Added it to 50 ml of warm ethanol and shaken vigorously to dissolve the solution was filtered through a tarred filter paper with 20 ml warm ethanol and dried it at 105 ˚C for one hour. The weight of dried paper was taken. [22]

Percentage alcohol insoluble matter = Weight of the residue×100 /Weight of sample

MOISTURE CONTENT

10g of soap sample was weighed immediately and recorded as "wet weight of sample". This wet sample was dried to a constant weight, at a temperature not exceeding 239º F (115º C) using the suitable drying equipment. The sample was cooled, weighed again and recorded as the "dry weight of sample". The moisture content of the sample was calculated using the following equation.[23]

$$\%W=100\ A- B/B \times 100$$

Where, %W = Percentage of moisture in the sample,

A= Weight of wet sample (grams),

B = Weight of dry sample (grams).

TOTAL FATTY MATTER (TFM)

TFM was estimated by reacting soap with acid in the presence of hot water and calculating the fatty acids obtained. 10g of the formulated soap was dissolved in 150 ml distilled water and heated. To this, 20 ml of 15% H2SO4 added while heating until a clear solution was obtained. Fatty acids that are present on the surface of the resulting solution are solidified by adding 7 g beeswax and heated again. Then, it was allowed to cake. Cake was removed and blotted to dry and weighed to obtain the TFM using the formula.[24]

% TFM= (Weight of the cake–Weight of the wax) in g/Weight of the soap in g×100.

ANTIFUNGAL STUDY

Antifungal assay using disc diffusion method. The fungal strains (from dandruff) were maintained on nutrient agar.A loopful of culture from the slant was inoculated into the

medium and incubated at 28°C for 48–72 hr, and 0.1 ml of this culture was evenly spread on the plates containing nutrient agar media. Sterile discs of Whatman No. 1 filter paper were impregnated on the surface of the media.10 µg/ml of different formulations (F1, F2, F3, F4), control (soap base),and standard (fluconazole) were prepared and applied on the discs and incubated for 48 h at 28°C. The results were recorded by measuring the inhibition zone around the discs.[25]

CLEANING EFFICIENCY BY THUMB IMPRESSION TEST

Thumbs of hands washed with the medicinal soap and the other thumb washed with the formulated soap was placed on the same nutrient agar medium plate separately and carefully without convergence of thumbprints. The behavior of microbial growth on the plates was observed after an incubation period of 24 h at 37°C.[26]

RESULT AND DISCUSSION

Collection and Authentication of Samples

Aqueous extract of stem and leaves of piper betel were used as the active ingredient for the study was collected from Kasaragod dist. Kerala. The samples were identified and authenticated by Dr. Subramanyam Prasad K, Professor, Nehru Arts and Science college, Kanhagad.

EVALUATION AND CHARACTERIZATION OF ANTIFUNGAL SOAP

The physicochemical parameters of the prepared soap were determined. Parameters such as colour, odour, appearance, pH were tested. The formulations exhibited good as appearance characteristic as well as the pH was found in the range 7.0 which is the desired pH. Other parameters such as, Foam height, Foam retention, Alcohol insoluble matter, and high temperature stability were determined and the results are tabulated.(TBLE 4)

PHYSICAL APPEARANCE

Physical appearance were studies and tabulated. (TABLE 5)

TOTAL FATTY MATTER (TFM)

TFM is the important characteristics describing the quality of soap. Here the TFM of fomulated soap is greater than 74. Higher TFM ensures that soap are least harmful to skin and not causes dryness.(FIGURE 1)

FOAM STABILITY AND FOAM RETENTION

From the above formulations foam height and foam retention of F4 was 10 ± 1.04 min and 25 ± 0.47cm.So it was found to be an effective formulation. (FIGURE 2)

ANTIFUNGAL ASSAY USING DISC DIFFUSION METHOD

Zone of inhibition of standard fluconazole drug – 13mm.

Zone of inhibition of F4 was found to be 12mm and was found to be effectively inhibit the fungal growth.

Refer FIGURE 3 & 4, TABLE 6 & 7.

CLEANING EFFICIENCY BY THUMB IMPRESSION TEST

Thumb impression were performed and there is no significant fungal growth on impression. (FIGURE 5)

Table 1: Formulation of base.

Ingredients	Quantity	Use
Coconut oil	10g	Antiaging, moisturizer
NaOH	1.77g	Lye
Distilled water	3.3ml	Aqueous vehicle

Table 2: Ingredients and Uses of Herbal Soap Formulation.

INGREDIENTS	USES
Piper betle	Antifungal
Aloe vera juice	Moisturizer, antifungal
Glycerin	Moisturizer
Perfume	Perfuming agent

Table 3: Formulation of Herbal Soap.

INGREDIENTS	QUANTITY			
	F1	F2	F3	F4
Piper betle	0.5g	1g	0.5g	1g
Aloe vera juice	0.5g	0.5g	1g	1g
Coconut oil	10g	10g	10g	10g
NaOH	1.7g	1.7g	1.7g	1.7g
Glycerin	1ml	1ml	1ml	1ml
Distilled water	3.33ml	3.33ml	3.33ml	3.33ml
Perfume	Qs	Qs	Qs	Qs

Table 4: Physicochemical Parameters of the Formulated Soap and Standard Synthetic Soap.

Formulation code	pH	Foam height (cm)	Foam retention (min)	Moisture content (%)	Alcohol insoluble Matter (%v/v)	TFM (%)
F1	7.1	22±1.04	8	3.3±0.12	18	74
F2	7.0±0.04	21±O.53	9±1.04	4.4±0.13	16	76
F3	7.1±0.04	23±0.47	9	3.7±0.18	16	76
F4	7.1	25±0.47	10±1.04	3.6	18	77
Standard (ketoconazole soap)	7.2	23	9±0.47	3.6	18	78

SD, n = 3

Table 5: Physical Appearance Parameters.

SL.NO	CHARACTERISTICS	OBSERVATION
1	Color	Light brown
2	Odor	Pleasant
3	Nature	Solid

Figure 1: TFM of Formulated Soap.

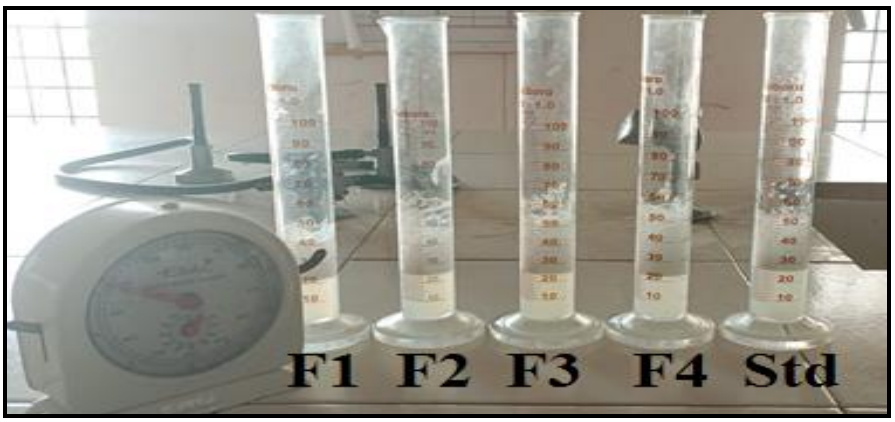

Figure 2: Foam Retention and Foam Height of Formulated Herbal And Synthetic Soap.

Figure 3: Antifungal activity of formulated soap F1, F2 on fungal strains: An in vitro comparative study.

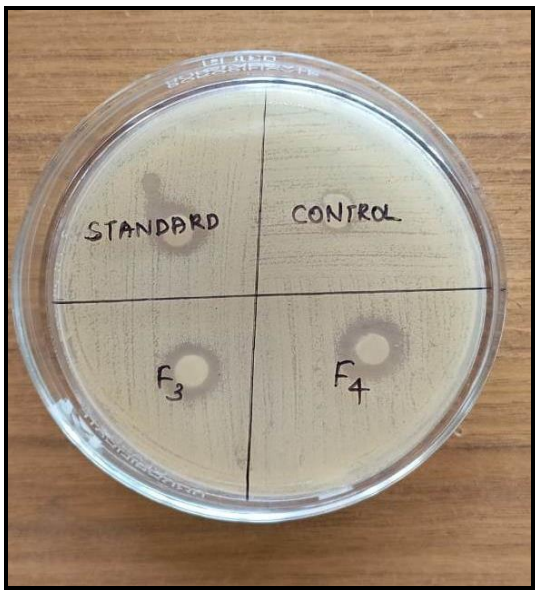

Figure 4: Antifungal activity of formulated soap F3, F4 on fungal strains: An in vitro comparative study.

Table 6: Zone of Inhibition of Different Formulations.

Formulations (10 µg/ml)		**Zone of inhibition**
Piper betel soap	F1	9mm
	F2	12mm
	F3	9mm
	F4	12mm
Control		5mm
Standard (fluconazole)		13mm

Table 7: Standard Sensitivity Range for Antifungal Drugs.

Antifugal drugs	Disc concentration	Zone of activity(mm)		
		sensitive	Intermediate SDD	Resistant
Fluconazole	10 µg/ml	≥20	12-19	≤ 11
Clotrimazole	10 µg/ml	≥19	15-18	≤ 14
Voriconazole	10 µg/ml	≥19	15-18	≤ 14
itraconazole	10 µg/ml	≥15	10-14	≤ 9

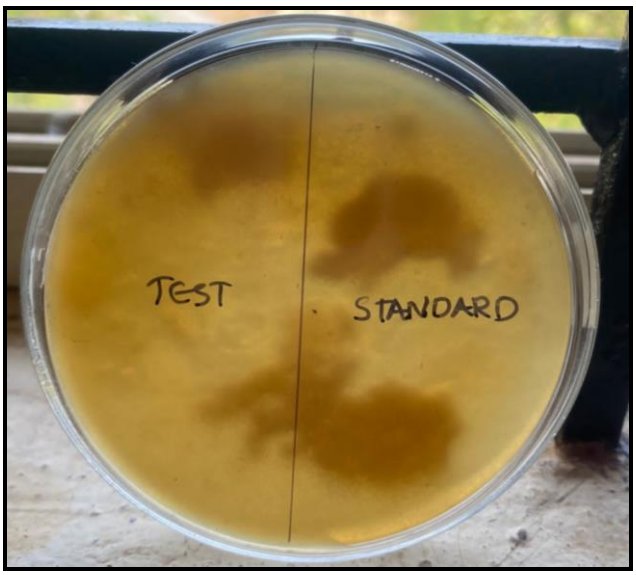

Figure 5: Thumb impression of test and standard formulation.

CONCLUSION

The Medicinal plants are considered as a rich source of various active constituents, which can be used in drug development and synthesis. Moreover some plants are considered as important source of nutrition and as a result of that these plants were recommended for their therapeutic values and the activities. We can use for the purpose of cosmetic formulation. Nowadays more dermatological diseases were reported due to pollution and other reasons. In our study we formulated an antifungal polyherbal soap for fungal infection on skin.

Different formulations (F1, F2, F3, and F4) of polyherbal soaps were successfully prepared and evaluated. The preformulation studies revealed that there was no interaction between drug and excipients. All the four formulations were prepared by cold process method and have obtained with proper consistency as same as normal soap. The physicochemical parameter of the prepared soap was determined. Parameters such as color, odor, appearance, pH were tested and satisfactory result were obtained. Antifungal study of all formulations were carried out by disc diffusion method and zone of inhibition of formulations, standard and control were measured.

Formulation F2 and F4 shown Maximum zone of inhibition and better antifungal activity. The formulation F4 also have a higher TFM value and it ensure that it does not produces irritation to skin and does not causes dryness. It also has highest foam height and foam retention time, so F4 was found to be stable and more active than all the other three formulations.

So, our formulation is capable for treating various skin diseases caused by fungus.

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