Antifungal and Phytochemical Screening of Extract from *Vitellaria Paradoxa* (Shea Butter Tree) Leaves, Barks and Roots on Dermatophytes

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Abstract- This study was carried out to investigate the phytochemical constituent and antifungal activities of extract from leaves, barks and roots of *Vitellaria paradoxa* (shea butter tree) against dermatophytes. The leaves, barks and roots of *Vitellaria paradoxa* were extracted using aqueous, ethanol and acetone as solvent of extraction. The extracts were tested for antifungal activities against dermatophytes using agar well diffusion and broth dilution method. The Phytochemical constituents of the extract of the plant parts were determined. The result of antifungal activities of the leaves, barks and roots of *Vitellaria paradoxa* indicated that there are significant differences ($p<0.05$) in the effect of the extracts when used at different concentrations. The largest zone of inhibition was recorded with ethanolic crude bark extract against *Microsporium audouinii* (20.5 mm), at 250mg/ml followed by acetone barks extract against *Microsporium audouinii* (19mm) at 250mg/ml. There was no activity with most of the aqueous extract except the bark extract. The lowest MIC/MFC value of 7.813/15.62mg/ml were obtained from ethanolic bark extract. This result has shown that the leaves, barks and roots extracts of *Vitellaria paradoxa* possess phytochemical constituents that can inhibit the growth of some dermatophytes and the bark extract proved to be most efficacious.

Index Terms- Antifungal, *Vitellaria paradoxa*, Phytochemical, Dermatophytes, Minimum Inhibitory Concentration and Minimum Fungicidal Concentration

I. INTRODUCTION

Dermatophytes are pathogenic fungi that specialize in infection of the skin, hair and nails that utilize keratinous substrates as the carbon, nitrogen and sulphur sources. They belong to three anamorphic (asexual or imprerfect) genera Epidermophyton, Microsporum and Trichophyton (Ellis *et al.*, 2000). They are spread by direct contact (anthropophilic organisms) animals (zoophilic organisms) and soil (geophilic organisms) as well as indirectly from formites (Barry and Hainer, 2003). It is one of the most common cutaneous infections world-wide and poses a great public health problem to human and animals (Ameen, 2010; Havlickova *et al.*, 2008). The spreading of this diseases in most developed countries of the world present both health and economic problem usually accompanied by a parallel increase in the infection of the nails (onycomyces) which are very difficult to treat (Seebacher *et al.*, 2008). In poor economic resource countries, mycoses appear endemically, primarily with children and their treatment often fail because of paucity of efficient antifungal (Ndako *et al.*, 2012).

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drug has been isolated from natural resources. Medicinal plants have been used for years in daily life to treat diseases all over the world (Ates and Erdogical, 2003). In African one of such plant which is claimed to have antimicrobial properties is shea butter tree (Vitellaria paradoxa) (Falana *et al.*, 2014).

The study of plant as antimicrobial is to address the problem of antimicrobial resistance by microbes (Odugbemi and Akinsulire, 2006). As majority citizens especially those that belong to low socio-economic group, use medicinal plant as complementary alternative medicine. It has become necessity that more scientific studies should be embarked upon to obtain scientific data that will provide benefit to humanity. Studies on medicinal plant highlight the potential clinical usefulness of plants in finding solution to health problems (Soladoye *et al.*, 2005; Odugbemi and Akinsulire, 2006). Therefore, the purpose of this work was to investigate the phytochemical constituent and antifungal efficacy of extract from leaves, barks and roots of *Vitellaria paradoxa* against *Trychophyton rubrum, Trychophyton mentagrophytes, Trychophyton schoeleinii, Microsporum audouinii and Microsporum ferugeneum*.

II. MATERIALS AND METHOD

2.1. Plant Collection and Identification

Fresh leaves, roots and barks of *Vitellaria paradoxa* were collected from the permanent site campus of the University of Ilorin, Ilorin. The plant samples were identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen number: UILH 001/961 was obtained for the sample.

2.2 Sample Preparation and Extraction Procedure

The fresh plant materials were air dried for a period of two weeks and they were pre-crushed in a mortar. They were later pulverized into fine powder using milling machine. A quantity (200g) of the fine powdered leaves roots and barks were weighed into a 2500ml capacity conical flask after which 1000ml of ethanol, sterile distilled water and acetone were poured respectively and allowed to stand for 48 hours with constant shaking at regular interval at room temperature. The percolates were then filtered and the solvent (Ethanol, Sterile distilled water and Acetone) were evaporated using rotary evaporator to obtain the ethanol, sterile distilled water and acetone extracts of the leaves, roots and barks respectively. These were stored in sterile air tight containers and stored in the refrigerator at 4ºC until needed for analysis.

2.3 Preparation of Dilution of Crude Extract for Antifungal Assay

The dry extracts were reconstituted with their respective solvents to give a concentration of 250mg/ml for the antifungal activity evaluation as described by (Banso and Ayodele, 2001).

2.4 Source and Identification of Test Organisms

The dermatophytes used in this study were *Microsporum audouinii, Microsporum ferugineum, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton schoenleinii.* They were obtained from the stock culture collection of Spectra Medic Diagnostic and Research Laboratories, Sagamu, Ogun State, Nigeria. The organisms were subcultured on Sabouraud Dextrose Agar containing 50mg Chloraphenicol, 400mg Cycloheximide and 40mg Gentamycin and incubated at ambient temperature (28-30ºC) for 1-7 days. The fungal isolates were reconfirmed using microscopic, cultural characteristic, growth rate and biochemical test as described by (Larone, 2002).

2.5 Preparation and Standardization of Inoculum

The preparation and standardization of inoculum was done according to the method described by Ogunledun *et al.,* (2008) with slight modification. Fungal spores of *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton schoeleinii, Microsporum ferugineum* and *Microsporum audouinii* were harvested after 7 days. The culture was flooded with 10ml of 40% sterile sucrose solution, suspension were made by gently scraping the colony with the tip of a sterile Pasteur pipette. The resulting suspended mixture was withdrawn and transferred to a sterile tube. Heavy particles of the suspension present were allowed to settle for 15 minutes at room temperature. The spores suspension were standardized to $1x10^6$ spores/ml by making 1:1000 dilution with sterile water to match an opacity of 0.5Mcfarland's standard.

2.6 Preparation of Sabouraud Dextrose Agar

65gm of sabouraud Dextrose Agar powder was weighed and dissolve in 1 liter of distilled water, then 400mg Cycloheximide, 50mg of Chloraphenicol and 40mg of Gentamycin were added to prevent saprophytic fungi and bacteria contaminant respectively. The media was autoclaved at 121ºC for 15minutes, and allowed to cool and dispensed into the petridishes. The media were allowed to solidify and dried in the oven at 45ºC.

2.7 Antifungal Susceptibility Test

2.71 Agar Well Diffusion Method

Sabouraud Dextrose Agar (SDA) was prepared according to specifications and autoclaved at 121ºC for 15minutes, and allowed to cool. The media was dispensed into sterile petri dishes and they were allowed to set and solidify. The media were dried in the oven at 45ºC before use. 1ml of standardized spores suspension containing $1x10^6$ spores/ml was evenly spread on the surface of the SDA plates and were allowed to dry. Then sterile cork borer (6mm in diameter) was used to make well equidistant to each other on each seeded plates and 0.1ml of the reconstituted aqueous, ethanol, and acetone extracts (250mg/ml, 125mg/ml and 62.5mg/ml) was dispensed into each labeled well. The solvent and standard drug fluconazole (50mg/ml) served as negative control and positive control respectively. The plates were allowed to stand on the bench for five minutes to allow the extract to diffuse into media and were incubated at ambient temperature for 1-7 days and observed for growth and all tests were done in duplicate. The zone of inhibition produced by the extract as well as the control were measured and recorded as means diameter of zones of inhibition around the wells (CLSI, 2006).

2.7.2 Determination of Minimum Inhibitory Concentration (MIC) of Extracts.

The minimum inhibitory concentration of the extracts was determined using broth dilution method as described by ibekwe *et al.,* (2001). Sabouraud Dextrose Broth was prepared according to the manufacturer's instruction and 1ml each was dispensed into separate test tube, sterilized at 121ºc for 15 minutes and then allowed to cool. Two fold serial dilutions of the extracts in the broth were made from the stock concentration of the extract to obtain concentrations of 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.81mg/ml, 3.91mg/ml and 1.59mg/ml. A 0.1ml of the standardized inoculums ($1x10^6$spores/ml) was inoculated into the different concentrations of the extracts in the broth. Controls which include water, 50% ethanol and 50% acetone were also set up along with the test experiment. All incubations were done at 30ºC for 1-7 days. The test tubes were observed for visible growth. The lowest concentration which showed no visible growth in the test tube was recorded as minimum inhibitory concentration.

2.7.3 Determination of the Minimum Fungicidal Concentration (MFC) of Extracts

Fresh Sabouraud Dextrose Agar media were prepared, sterilized at 121ºC for 15 minutes, allowed to cool, poured into sterile petri dishes and left to solidify. The content of the tube with the minimum inhibitory concentrations were sub cultured onto the media. All the plates were incubated at 30ºC for 1-7 days and observed for growth. The Minimum Fungicidal Concentration was taken as the lowest concentration of the extracts without growth on the agar plate.

2.8 Statistical Analysis

The data obtained from the study were analyzed statistically using the Analysis of Variance (ANOVA) in Statistical Package for Social Sciences (SPSS) version 21.00.

2.9 Phytochemical Screening

The aqueous, ethanol and acetone extracts of various parts (leaves barks roots) were prepared by dissolving 2g of each extract

in 20ml of solvents used and were analysed for the presence of Alkaloid, Tannins, Saponins, Flavonoids steroids, cardiac glycosides phenolics, Phlobatanin and carbohydrates as described by Trease and Evans (2002).

III. RESULTS AND DISCUSSION

Many naturally occurring compounds found in plants, herbs and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens (Kumar *et al*., 2006).

The preliminary phytochemical screenings of leaves, barks and roots of *Vitellaria paradoxa* (Acetone, ethanol and water) extracts revealed the presence of saponins, tannins, alkaloids, flavonoids, phlobotanins and phenolic compounds in the extracts. The result from this study showed that the ethanolic extract of the plant parts contain all the phytoconstituents tested except saponin in leaf and root. Of the three solvents used, saponin was detected in the aqueous extracts of the three plant parts investigated. The aqueous extract did not contain phlobatannin, glycosides and carbohydrate in all the three parts and alkaloid in the bark and root extract while acetone extract did not contain carbohydrate in all the three plant parts and saponin in leaf and root. Other phytoconstituents tested were detected in acetone extract of all three plant parts except glycosides in bark (Table 3.1).

These secondary metabolites are known to be biologically active and play significant roles in bioactivity of medicinal plants, because the medicinal values of plant lies in these phytochemical compounds which produce a definite and specific action on the human body. Saponins, a special class of glycosides with soapy characteristic, were reported as active antifungal agents (Ogu *et al.,* 2011). Tannins have been reported to hinder the development of micro-organisms by their ability to precipitate and inactivate microbial adhesions enzymes and cell envelope proteins (Ogu *et al.,* 2011; Cowan, 1999). The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with microbial cell wall, thereby disrupting their membrane integrity (Ogu *et al.,* 2011). The significant antifungal activities observed in this study could thus be attributed to the interaction of one or more of the identified metabolites against the dermatophytes.

The result of antifungal activities of the leaves, barks and roots of *Vitellaria paradoxa* indicated that there were significant differences ($p<0.05$) in the effect of the extracts when used at different concentrations, and as well the level of activities among the parts of the plant. However, the highest zone of inhibition was produced when higher concentration (250mg/ml) was used. Ethanolic extracts of the leaves, roots and barks produced the highest inhibition zones (Table 3.2), followed by acetone extracts (Table 3.3) and the lowest inhibition zone was produced by aqueous extracts used at high concentration. No inhibition was produced when they were used at concentrations lower than 250mg/ml (Table 3.4).

Among the dermatophytes, the largest zones of inhibition were recorded with ethanolic crude bark extract against *Microsporium audouinii* (20.5mm), *Microsporium ferrugenum* (19.5mm) and *Trichophyton rubrum* (17.5mm) followed by acetone bark extract against *Microsporium audouinii* (19mm), *Microsporium ferrugenum* (17.5mm) and *Trichophyton rubrum* (16.5mm) at 250mg/ml. The antifungal activities of the crude extracts of roots and leaves also showed significant zones of inhibition and the aqueous extract has no activity except for the bark extract that showed narrow activity against *Microsporium audouinii* (6mm) *and Microsporium ferrugenum* (5.5mm) at the highest concentration of 250mg/ml. This result is in agreement with the findings of Ahmed et al., (2009) who reported that the bark extract of *Vitellaria paradoxa* was active against some dermatophytes. The antimicrobial activity of the plant part varies with the solvent of extraction, ethanol extracts have the highest activity. Falodun *et al.,* (2006) and Nsor-Atindana *et al.,* (2012) have reported that solvent of extraction is the major determinant of antimicrobial activity of plant extracts. Differences in polarity among various solvents have been reported to be accountable for the differences in solubility of plant active principles, hence variation in degree of activity (El-Mahmood *et al.,* 2008). The water extracts however, demonstrated no activity against all the tested dermatophytes. When plant materials are grounded in water or the plant cells are damaged, some phenolases and hydrolases are often released and these enzymes might have modulatory effect on the activity of the active compounds in the extract or there may be incomplete extraction of the active principles thus explaining the low activity (El-Mahmood *et al.,* 2008).

The low MIC/MFC values of 7.813/15.62mg/ml were obtained for ethanolic bark extract against *Tricophyton rubrum, Trichophyton schoeleinii, Microsporum ferrugenum* and *Microsporium audouinii and* 15.62/31.25mg/ml against *Trichophyton mentagrophyte*. Also the ethanolic root extract has MIC/MFC value of 7.813/15.62mg/ml against *Microsporum ferrugenum* and *Microsporium audouinii* and MIC/MFC value of 31.25/62.5mg/ml against *Trichophyton mentagrophyte Trichophyton rubrum and Trichophyton schoeleinii*. The ethanolic leave extract has MIC/MFC value of 15.62/31.25mg/ml against *Microsporium audouinii* and MIC/MFC value of 31.25/62.5mg/ml against *Tricophyton rubrum, Trichophyton schoeleinii, Microsporum ferrugenum* and *Trichophyton mentagrophyte* (Table 3.5). The MIC/MFC value of acetone extract were similar to that of ethanolic extract of leave, bark and root(Table 3.6) except the aqueous extracts that have MIC/MFC value of >250/>500mg/ml against all the dermatophytes (Table 3.7). Ahmed et al., (2009) reported that agent with low antimicrobial activity against an organism would require high concentration MIC/MFC while those with high activity require low concentration to either inhibit or totally kill such organism.

The MIC and MFC values obtained showed that the ethanolic extracts of *Vitelleria paradoxa* has the most potent effect against tested organisms and the bark extract of *Vitellaria paradoxa* could possess potent fungicidal components against the tested dermatophytes at very low concentration than the root and the leave extracts. The MIC and MFC results showed that the extracts exhibited definite fungistatic and fungicidal activities. This result signifies the probable optimum concentration of such extracts that could inhibit or cause a total cidal effect on the tested organisms (Brooks *et al.,* 2003).

IV. CONCLUSION

In conclusions, this result has shown that the leaves, barks and roots extracts of *Vitellaria paradoxa* possess Phytochemical

constituents that can inhibit the growth of some dermatophytes. It was also observed from this work that ethanol and acetone were better for extraction because the bioactive substances of all the parts of *Vitelleria paradoxa* are more soluble in those solvent than water. Of the three plant parts of *Vitellaria paradoxa* (leaves barks and roots), the bark extract proved to be most efficacious.

Table 3.1: Phytochemical Analysis of Leaves, Barks and Roots Extracts of *Vitellaria paradoxa*

Phyto consistituent	Aqueous extract Leaves /Barks/Roots	Ethanol extract Leaves /Barks/Roots	Acetone extract Leaves /Barks/Roots
Saponin	+ + +	- + -	- + -
Alkaloids	+ - -	+ + +	+ + +
Flavonoids	+ + +	+ + +	+ + +
Tannin	+ + +	+ + +	+ + +
Steroids	+ + +	+ + +	+ + +
Phenols	+ + +	+ + +	+ + +
Phlobatannin	- - -	+ + +	+ + +
Glycoside	- - -	+ + +	+ - +
Carbohydrate	- - -	+ + +	- - -

+ = present, - = absent, + + + = present in leave, bark and root extracts

3.2: Comparative Antidermatophytic activities of the ethanolic extract of *Vitelleria paradoxa*

Org	50% ETH	Zones of inhibition (mm) 0f various Concentration of Ethanolic extracts(mg/ml)								
		50mg/ml		62.5mg/ml		125mg/ml			250mg/ml	
		Flu	LEAF	BARK	ROOT	LEAF	BARK	ROOT	LEAF	BARK
TR	0±0.0	10.2±1.11	2.1±0.27	6.0±2.66	3.0±1.00	3.5±0.17	10.0±1.00	7.5±0.50	6.0±1.00	17.5±1.32
TM	0±0.0	12.9±0.46	2.6±0.40	5.0±1.00	2.5±0.53	3.8±0.27	9.0±1.00	5.0±1.00	5.0±2.00	13.5±1.32
TS	0±0.0	11.6±0.56	3.1±0.10	5.5±0.87	3.0±1.00	5.3±0.30	9.8±0.27	6.0±2.66	8.0±1.73	14.0±4.36
MF	0±0.0	17.0±0.92	3.6±0.20	6.5±0.20	4.0±1.00	5.4±0.30	10.0±1.18	8.0±2.65	8.0±1.73	19.5±1.80
MA	0±0.0	15.6±1.49	3.8±0.53	9.0±1.73	4.5±0.56	7.0±0.92	12.0±1.00	10.0±3.22	9.0±2.00	20.5±1.32
F - Value		17.636		9.627			9.583			15.794
P-Value		<0.05		<0.05			<0.05			<0.05

TR = *Trichophyton rubrum*, TM = *Trichophyton mentagrophyte*, TS = *Trichophyton schoeleinii*, MF = *Microsporium ferrugenum*, MA = *Microsporium audouinii*, ETH=Ethanol, FLU = Fluconazole

Table 3.3: Comparative Antidermatophytic activities of the Acetone extract of *Vitelleria paradoxa*

Org	50% ETH	Zones of Inhibition (mm) of various Concentration of Acetone Extracts(mg/ml)								
		50mg/ml		62.5mg/ml		125mg/ml			250mg/ml	
		Flu	LEAF	BARK	ROOT	LEAF	BARK	ROOT	LEAF	BARK
TR	0±0.0	10.2±1.11	2.0±0.0	5.6±0.6	3.0±1.0	3.5±1.1	9.1±0.8	7.0±0.8	5.5±0.6	16.5±2.1
TM	0±0.0	12.9±0.46	1.6±0.3	4.5±0.4	2.3±0.3	2.5±0.7	8.0±2.7	3.8±0.4	4.0±1.1	11.0±4.6
TS	0±0.0	11.6±0.56	3.0±1.0	5.0±1.0	2.0±0.3	6.3±0.5	9.6±0.6	3.6±0.6	10.0±1.0	13.0±2.0
MF	0±0.0	17.0±0.92	2.8±0.2	6.5±0.2	4.3±0.4	4.4±0.6	10.0±3.6	8.9±0.8	6.0±1.0	17.5±1.5
MA	0±0.0	15.6±1.49	3.2±0.7	8.9±0.8	5.0±1.1	7.0±1.0	12.0±1.7	9.0±0.4	9.0±1.7	19.0±2.0
F - Value		17.636		28.440			12.893			19.690
P-Value		<0.05		<0.05			<0.05			<0.05

TR = *Trichophyton rubrum*, TM = *Trichophyton mentagrophyte*, TS = *Trichophyton schoeleinii*, MF = *Microsporium ferrugenum*, MA = *Microsporium audouinii*, ACE= Acetone , FLU = Fluconazole

Table 3.4: Comparative Antidermatophytic activities of the Aqueous extract of *Vitelleria paradoxa*

Org	50% ETH	Zones of inhibition (mm) of various Concentration of Aqueous extracts(mg/ml)								
		50mg/ml		62.5mg/ml		125mg/ml			250mg/ml	
		Flu	LEAF	BARK	ROOT	LEAF	BARK	ROOT	LEAF	BARK
TR	0±0.0	10.2±1.11	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
TM	0±0.0	12.9±0.46	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
TS	0±0.0	11.6±0.56	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
MF	0±0.0	17.0±0.92	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	5.5±0.63
MA	0±0.0	15.6±1.49	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	4.0±1.0	6.0±2.65
F - Value		17.636								
P-Value		<0.05								

TR = *Trichophyton rubrum*, TM = *Trichophyton mentagrophyte*, TS = *Trichophyton schoeleinii*, MF = *Microsporium ferrugenum*, MA = *Microsporium audouinii*,WAT=Water,FLU=Fluconazole

Table 3.5: MIC/MFC of the Ethanolic extract of *Vitelleria paradoxa*

ORG	MIC (mg/ml)				MFC (mg/ml)			
	LEAVE	BARK	ROOT	ETH	LEAVE	BARK	ROOT	ETH
TR	31.25	7.813	31.25	0.00	62.5	15.625	62.5	0.00
TM	31.25	15.625	31.25	0.00	62.5	31.25	62.5	0.00
TS	31.25	15.625	31.25	0.00	62.5	31.25	62.5	0.00
MF	31.25	7.813	7.813	0.00	62.5	15.625	15.625	0.00
MA	15.625	7.813	7.813	0.00	31.25	15.625	15.625	0.00

TR = *Trichophyton rubrum*, TM = *Trichophyton mentagrophyte*, TS = *Trichophyton schoeleinii*, MF = *Microsporium ferrugenum*, MA = *Microsporium audouinii*, ETH =Ethanol

Table 3.6: MIC/MFC of the Acetone Extract of *Vitelleria paradoxa*

ORG	**MIC (mg/ml)**			**MFC (mg/ml)**			
LEAVE	BARK	ROOT	ACE	LEAVE	BARK	ROOT	ACE
TR	31.25	15.625	31.25	0.00	62.5	31.25	62.5	0.00
TM	31.25	15.625	31.25	0.00	62.5	31.25	62.5	0.00
TS	31.25	15.625	31.25	0.00	62.5	31.25	62.5	0.00
MF	31.25	7.813	15.625	0.00	62.5	15.625	31.25	0.00
MA	15.625	7.813	7.813	0.00	31.25	15.625	15.625	0.00

TR = *Trichophyton rubrum*, TM = *Trichophyton mentagrophyte*, TS = *Trichophyton schoeleinii*, MF = *Microsporium ferrugenum*, MA = *Microsporium audouinii*, ACE=Acetone

Table 3.7: MIC/MFC of the Aqueous extract of *Vitelleria paradoxa*

ORG	MIC (mg/ml)				MFC (mg/ml)			
	LEAVE	BARK	ROOT	WAT	LEAVE	BARK	ROOT	WAT
TR	>250	>250	>250	0.00	>500	>500	>500	0.00
TM	>250	>250	>250	0.00	>500	>500	>500	0.00
TS	>250	>250	>250	0.00	>500	>500	>500	0.00
MF	>250	>250	>250	0.00	>500	>500	>500	0.00
MA	>250	>250	>250	0.00	>500	>500	>500	0.00

TR = *Trichophyton rubrum*, TM = *Trichophyton mentagrophyte*, TS = *Trichophyton schoeleinii*, MF = *Microsporium ferrugenum*, MA = *Microsporium audouinii*, WAT= Water

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