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Phytochemical Profiling and FTIR Characterization of Hyptis suaveolens (L.) Poit

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Abstract

Hyptis suaveolens (Syn. Mesosphaerum suaveolens) is an aromatic plant species from lamiaceae family originated in the tropical regions of Mexico, South America, and the West Indies. It is naturalized in tropical parts of Asia, Australia and Africa and now considered as an obnoxious invasive species. The plant has been traditionally used for its antimicrobial, anti-inflammatory and wound healing properties. This study aims to investigate the phytochemical composition and functional groups of H. suaveolens using qualitative phytochemical screening and FTIR. Fresh leaves were collected; shade dried, powdered and subjected to methanolic extraction using soxhlet apparatus. The powdered samples were analyzed to document the organoleptic characters. The powered samples were green with minty odour, bitter taste and fine texture. Colour changes in reaction to various chemicals/reagents indicated the presence of various secondary metabolites. Phytochemical screening revealed the presence of phenols. terpenoids/steriods etc.

FTIR is an effective tool to identify the therapeutic compounds and enhancing quality assurance. It provides detained chemical fingerprinting. Key absorption peaks in powdered sample as well as in methanolic extracts observed for functional groups such as O-H (phenols/flavonoids), C-H (alkanes/terpenoids), C=C or C=O (aromatic rings), and C-O/).C (ethers/esters). These confirmed the presence of phyto-compounds viz., 1,8-Cineole, Limonene, β-caryophyllene, Sabinene, Quercetin derivatives, and phenolic acids like caffeic acid. The comparative study revealed the presence of higher phenolic content in the powdered sample than in methanolic extract. This comprehensive profiling supports the medicinal importance of this plant aligning with its traditional uses. The investigation indicate significant potential for pharmaceutical and cosmetic applications, warranting further isolation and in vivo validation of its bioactive constituents

Key Words: Hyptis suaveolens, FTIR, phenolic compounds, terpenoids, leaves, phytochemical screening

Introduction

India is endowed with almost 10% of biodiversity wealth of the world and many are of with medicinal properties Even in the age-old herbal therapy record 'Chraka Samhita' highlights the significance of herbal therapy using various medical formulations. In the developed world, herbal medicines are of great demand for the primary health care due to their safety, efficacy and lesser side effects. In this sector, it is estimated that about one fourth of the drugs are plant derived, while rest are synthetic analogue based on prototype compounds isolated from plants. In certain age-related diseases for which there is no modern medicine is available till date, the herbs offer therapeutics. These are attributed by the plants through the chemical compounds produced by the plant itself that is secondary metabolites. The active ingredients of many medicines have been identified and their usefulness in drug therapy is being determined. The over-exploitation of plants from the wild may lead to substantial loss of their habitat and an unpredictable decline in it population. Bush Min (*Hyptis suaveolens)* is an invasive weed with numerous phyto-compounds, signifies its potentiality to cure ailments. Traditionally the plant is valued its wound healing, antimicrobial, and anti-inflammatory properties (Danmalam et al., 2009). In order to find out its role in medication, preliminary phytochemical screeening and subsequent phytochemical analysis is necessary. Considering these, we devoted our effort to find out the phytochemicals present in the leaf and the methanolic leaf extracts in a qualitative manner. Further, Fourier Transform Infrared (FTIR) spectroscopy was also done to get a molecular fingerprint of the crude drug and the methanolic extract.

Materials and Methods

Collection of plant materials

Healthy twigs of Hyptis suaveolens were collected from the nearby areas of St. Thomas College, Ranni, and were positively identified and confirmed.

Preparation of plant samples

The fresh leaves of H. suaveolens were washed in distilled water. They were then shade dried for 7-8 days. With the help of mortar and pestle, the dried materials are powdered and these raw drugs were used for further

analysis. 10 grams of the powdered samples were subjected to hot extraction using a Soxhlet apparatus. The extracts were collected in a clean petridish, and methanol was evaporated to get the powdered extract, which was used for further investigations

Phytochemical Analysis

Shade dried, powdered raw drug was used for analysis, according to methods described by Alamgir and Alamgir (2017).

Organoleptic characters: The crude drug samples were analysed to identify the organoleptic characters through the sense organs. These include colour, odour, and taste. The samples were placed in the palm of the hand and crushed the sample using gentle pressure and slowly and repeatedly inhale the air over the material. The taste of the samples was identified with the help of our tongue. By touching, the fibrous, rough, granular, nature can be identified. The size can be identified with the pore size of the sieve, which is used for this purpose.

Behaviour of Hyptis suaveolens with different reagent/solvents

The characteristic changes of the drug in presence of different solvent/reagent were identified. The behaviour of powder/ extract under different regimes of wavelength of lights was also identified to standardize the purity of the drug sample.

Qualitative phytochemical analysis

Phytochemical screening was performed to determine the class of phyto-constituents present in the drug. The chemical tests were carried out on the methanol extract of procedure as described by Harborne (1988), Daniel and Daniel (1991). FTIR analysis was done to confirm the functional group.

Result and Discussion

Hyptis suaveolens is an invasive species usually found along roadside, but is noticed for its thulsi like smell. Phytochemical studies of the leaf in powered and extracted form have bought many unknown information and conformations are described and discussed here.

Powder Analysis

The organoleptic characters and different classes of phytochemicals in the leaf powder were analyzed. The characters identified through sense organs are called organoleptic characters. The powder of the species looks Misty grey. The texture is considered the fiber. The taste is bitter (Table 1). The characters studied were the colour, odour, size, texture, and taste of the

Character	Leaf powder
Colour	Green
Odour	Minty smell
Size	Moderately
Texture	Bitter
Taste	Fine

Table 1: Organoleptic characters of Hyptis suaveolens

Phytochemical properties of powdered sample

The crude drug reacts uniquely with different reagents. In the present study the behavior of powdered samples in different reagents and the colour identified with the color chart of Asian paints. The colour formation is in these drugs are due to the metabolites present in it and this can be considered as a way to identify the purity of the drug (Table 2). The powder analysis revealed that under all chemicals reacts and produce different color. The results were further studied under long and short UV and Fluorescent light. The result is summarized in table 3. The reaction of methanolic extract under different chemicals/reagents were also done and depicted in table 4.

Table 2: Behaviour of powdered sample in the presence of different chemicals

Reagent	Colour
Powder + distilled water	Sand
Powder + 5% KOH	Sepia
Powder + Acetic acid	Yellowish brown
Powder + Conc. HCl	Pale blueish
Powder + Conc.H_2SO_4	Essence
Powder + N/10 Iodine	Sunderban
Powder + Ammonia sol.	Dark brown

Powder + 5% NaOH	Sepia
Powder + HNO_3	Rosy brown
Powder + $FeCl_3$	Chocolate

Table 3.Behavior of powdered samples in short UV light, long UV light and visible light

Treatment	Long UV	Short UV	Visible light
Powder alone	Green	Black	Green
Powder + H_2SO_4	Inchworm green	Off black	Lemon yellow
Powder + HNO_3	Spring green	Colourless	Light tan
Powder + HCl	Green	Charcoal	Camel
Powder + 10ml HCl	Colourless	Dim gray	Spring bouquet
Powder + KOH	Juniper	Cinnamon	Copper
Powder + NaOH	Bright Yellowish green	Peanut bran	Medallion

Phytochemical analysis

The methanol extract were screened qualitatively for identified the occurrence of phytochemicals. The other phytochemical analysis like alkaloids, anthraquinones, carbohydrates, glycosides, phenols, tannins, saponines, steroids/terpenoids were identified were added in the table 5. The medicinal properties of the plant is because of the presence of phyto-compounds present in them. In the study it was revelaed that the methanolic extract contain phenol and steroid/tepenoids. Many phenols are brightly coloured pigments and are responsible for the colouration in the and other tissues. The phenolic compounds are actively involved in the defence of the plants against invading pathogen including bacteria, fungi and viruses. Its presence indicates the antimicrobial property of these species. In plants terpenoids/steroids help to resists biotic and abiotic stress condition and play a major role in the management of cardio-vascular and cerebro-vascular disorders.

Table 4: Behavior of methanol extract in presence of different reagent/solvents

TREATMENT	Colour formed
Extract + distilled water	Light dirty gold
Extract + $FeCl_3$	Cyber yellow
Extract + Acetic acid	Lemon
Extract + KOH	Summer daffodil
Extract + HCl	Flax
Extract + NaOH	Summer daffodil
Extract + H_2SO_4	Creamy corn
Extract + N/10 Iodine sol.	Champaign
Extract + Ammonia sol.	Hazelnut
Extract + HNO_3	Pantone

Table 5: Qualitative screening of phytochemicals

Phytochemicals	Methanol extract
Alkaloids	-
Anthraquinones	-
Carbohydrates	-
Glycosides	-
Phenol	+
Tannins	-
Saponins	-
Steroids/ Terpenoids	+

The qualitative estimation phyto-compounds by using various reagents will give an idea about the chemical profile of the plant species (Table 5). In the present study it was revealed that H.suaveolens contain alkaloids, carbohydrates, flavonoids, phenols, steroids and terpenoids, which is in conformation with earlier work (Prabhat et al., 2009).

FTIR analysis

Fourier Transform Infrared spectroscopy helps to assess the chemical profiling of a plant species. FTIR has a pivotal role in phytochemical investigation. In this study the FTIR of both crude drug and methanolic extracts was done to identify the compounds present in the plant. The analysis was done at STIC, Kerala. The plant has been traditionally used for wound healing and exhibits notable antimicrobial and anti-inflammatory properties, attributed to various secondary metabolites such as flavonoids, phenolic compounds, tannins, and terpenoids

(Figure 1 and 2). Based on the FTIR spectrum provided, the peaks can be interpreted and identify the phytochemicals. FTIR analysis provides a rapid, non-destructive method for the identification of characteristic functional groups. Likely Bioactive Compounds in Hyptis suaveolens (based on FTIR profile) can be summarized as follows

1,8-Cineole (Eucalyptol): Since the peaks at ~1100–1250 cm^{-1} (C–O–C), 2900 cm^{-1} (C–H) the groups present have ether (C–O–C), cyclic hydrocarbon (C–H), weak O–H; β-Caryophyllene (sesquiterpene): Since the peaks at ~1646 cm^{-1} (C=C), ~1460 cm^{-1} (C–H bending), the groups present have Alkene (C=C), methyl (C–H), cyclobutene; Limonene / Sabinene (monoterpenes): Peaks at ~2930–2970 cm^{-1} (C–H stretch), ~1646 cm^{-1} (C=C) indicate the presence of alkene (C=C), methyl groups; Flavonoids (e.g., Quercetin derivatives): Broad O–H (~3449 cm^{-1}), C=O/C=C (~1646 cm^{-1}), aromatic bends (~800–900 cm^{-1}) identifies group with multiple O–H, C=O (conjugated), aromatic rings; Coumarins or Phenolic acids (e.g., caffeic acid): Peaks between 1100–1270 cm^{-1} and aromatic regions indicate the presence of aromatic ring, hydroxyl, ether or ester.

Table 6: Major peaks and their assignments in the methanolic extract of H. suaveolens

Wavenumber	Vibration	Functional Group
~3449	O–H stretching (broad)	Phenols or alcohols (flavonoids, terpenoids)
~2932–2970	C–H stretching	Alkanes (in terpenes like limonene, sabinene)
~1646	C=C or conjugated C=O stretch	Aromatic rings, flavonoids, terpenoids
~1466, ~1379	C–H bending (scissoring)	Alkanes (methyl/methylene groups)
~1341	O–H bending or NO_2 stretch	Phenols or nitro groups
~1279–1230	C–O stretch	Ethers, esters, phenols (cineole, flavonoids)
~1160–1107	C–O–C / C–N stretch	Ethers and amines
~1030–1070	C–O stretch	Alcohols, ethers (flavonoids, essential oils)
~800–900	Aromatic C–H out-of-plane bend	Aromatic compounds (e.g., flavonoids)

The FTIR spectrum supports the presence of 1,8-Cineole, β-Caryophyllene, Limonene or Sabinene, Flavonoids (likely derivatives of quercetin or kaempferol), and Phenolic compounds (e.g., coumarins, caffeic acid). These compounds are consistent with *H. suaveolens* essential oil and extract composition, known for antimicrobial, antioxidant, and anti-inflammatory properties.

Compounds in Crude Leaf Powder of *Hyptis suaveolens* are

1,8-Cineole with peak at ~1230–1279 (C–O–C), 2930–2970 (C–H stretch) and the functional group observed are ether (C–O–C), C–H (cyclic), mild O–H; Sabinene: peak is at~1647 (C=C), 2930–2970 (C–H), 1466 (CH_2 bend) having Alkene (C=C), methyl/methylene (C–H) as functional groups; Limonene: the peak is at~1647, 2930–2970, ~1379 indicating Alkene (C=C), methyl (C–H) as functional groups; β-Caryophyllene: since peak at ~1647 (C=C), ~1466, ~1379, the functional groups identified were Cyclobutane, alkene, methyl; Quercetin, Flavonoids: The functional groups are O–H, C=O, aromatic rings the peak was at~3449 (O–H), ~1647 (C=O/C=C), 800–900 (C–H bend); Phenolic acids(e.g., caffeic acid): since the peak is ~3449 (O–H), ~1647, 1230–1100 and the functional groups are O–H, C=O, aromatic C=C. Coumarins: since the peak noticed at ~1647, ~1230–1279, 800–900 having Aromatic rings, esters, C=O as functional groups. This is in confirmation with earlier work by Joseph and Jeeva (2016).

Table 7: Major FTIR peaks and their assignments in the powdered leaf of H.suaveolens

Wavenumber (cm^{-1})	**Functional Group**	**Probable Source Compound**
~3348	O–H stretch (broad)	Flavonoids, phenols (e.g., quercetin)
~2970, 2932	C–H stretch (alkanes)	Terpenoids (sabinene, limonene, etc.)
~1647	C=C or conjugated C=O	Aromatic rings or α,β-unsaturated ketones
~1466, ~1379	C–H bending	Terpenoids or methyl/methylene groups
~1341	O–H bend / NO_2 symmetric	Phenols / secondary metabolites
~1279–1230	C–O stretch	Flavonoids, esters, coumarins
~1160, ~1107	C–O–C stretch / ethers	Essential oil components (e.g., cineole)
~1030–1070	C–O–C stretch (ethers)	Terpenoids (cineole), flavonoids
~800–900	Aromatic C–H bending	Aromatic rings in flavonoids / coumarins

The inclusion of FTIR analysis in phytochemical research not only supports the validation of traditional medicinal claims but also contributes to the standardization and quality assurance of plant-based products. Thus,

integrating FTIR into the phytochemical profiling is essential for advancing pharmacognostic research, ensuring reproducibility, and meeting regulatory requirements in natural product development.
In general we can say the phytochemicals present are as follows: Broad O–H (~3449 cm^{-1}): Indicates polyphenols/flavonoids, C–H stretch (~2970–2932 cm^{-1}): Aliphatic chains (terpenoids), C=C and C=O (~1647 cm^{-1}): Aromatic rings and/or conjugated ketones, C–H bending (~1466, ~1379 cm^{-1}): Alkanes in essential oil components, C–O and C–O–C (~1279–1030 cm^{-1}): Ethers, esters (cineole, flavonoids), Aromatic out-of-plane bend (~800–900 cm^{-1}): Aromatic compounds (flavonoids, coumarins).

Table 8: FTIR Comparison of Hyptis leaf powder and methanolic leaf extract

Wavenumber	Extract	Powder	Assignment
~3449	Strong, broad O–H	Similar O–H stretch	Phenols or alcohols (flavonoids, etc.)
~2970–2932	Present	Present	C–H stretching (alkanes, terpenoids)
~1646–1647	Present	Present	C=C or conjugated C=O (aromatics/flavonoids)
~1466, ~1379	Present	Present	CH_2/CH_3 bending (aliphatic chains)
~1341	Moderate	Stronger	O–H bending / NO_2 (phenols or nitro compounds)
~1279–1230	Strong	Strong	C–O stretching (ethers, esters, phenols)
~1160–1107	Clear	Clear	C–O–C or C–N (ethers, essential oils)
~1030–1070	Distinct	Distinct	C–O (ethers, alcohols, terpenoids)
~800–900	Moderate (aromatic ring bending)	Stronger	Aromatic C–H out-of-plane bend

The comparison revealed that the O-H intensity is strong in both samples. The C-O region (1230-1279cm^{-1}) was found to be strong in both samples but likely rich in flavonoids/ethers in extract, while in powder it is consistent. The aromatic region (800-900 cm^{-1}) is moderate in extract while stronger in powder, suggesting the presence of more flavonoids or phenols. More intense at 1341 cm^{-1} region indicates the richer phenolic content in crude form of drug.

Summary and Conclusion

Hyptis suaveolens, an aromatic and invasive plant often found along roadsides, has been examined for its phytochemical constituents and functional groups using both powdered and methanolic extract forms. Despite its invasive nature, the plant emits a characteristic minty or Tulsi-like odour and is recognized for its traditional use in wound healing and antimicrobial applications.

Powder Analysis:

- Organoleptic assessment described the powder as green in colour with a minty odour, bitter taste, and fine texture.
- Upon reaction with various chemical reagents, the powder exhibited distinct colour changes, indicating the presence of different metabolites.
- Under visible, short UV, and long UV light, unique fluorescence and colour shifts confirmed the variability in compound presence and quality markers.

Phytochemical Screening:

- Methanolic extracts were screened qualitatively, confirming the presence of phenols and terpenoids/steroids, while alkaloids, glycosides, saponins, and carbohydrates were absent.
- Colorimetric reactions with chemical reagents suggested diverse classes of secondary metabolites in the extract.

FTIR Analysis:

- FTIR spectra of both methanolic extract and crude powder confirmed the presence of key functional groups such as O–H (phenols/flavonoids), C–H (alkanes/terpenoids), C=C or C=O (aromatic rings, flavonoids), C–O and C–O–C (ethers, esters, essential oils), and aromatic ring bending (flavonoids, coumarins).
- Identified compounds likely include:
  - 1,8-Cineole, Limonene, Sabinene, and β-Caryophyllene (terpenoids and essential oil components)
  - Quercetin, Kaempferol, and other flavonoids
  - Phenolic acids such as caffeic and ferulic acids
  - Coumarins

The comprehensive phytochemical and FTIR analyses confirm that *H. suaveolens* is rich in bioactive compounds. These constituents contribute to its documented medicinal properties like antimicrobial, antioxidant, and wound healing activity. FTIR spectroscopy effectively validated these compounds, demonstrating its utility in the standardization a quality control of herbal formulations. Given its phytochemical richness, *H. suaveolens* has significant potential for use in traditional medicines, and cosmetics, despite its status as an invasive species. Future work should focus on isolating individual bioactive compounds and validating their pharmacological effects through in vivo studies.

Figure 1: FTIR spectra of Hyptis suaveolens Methanolic leaf extract

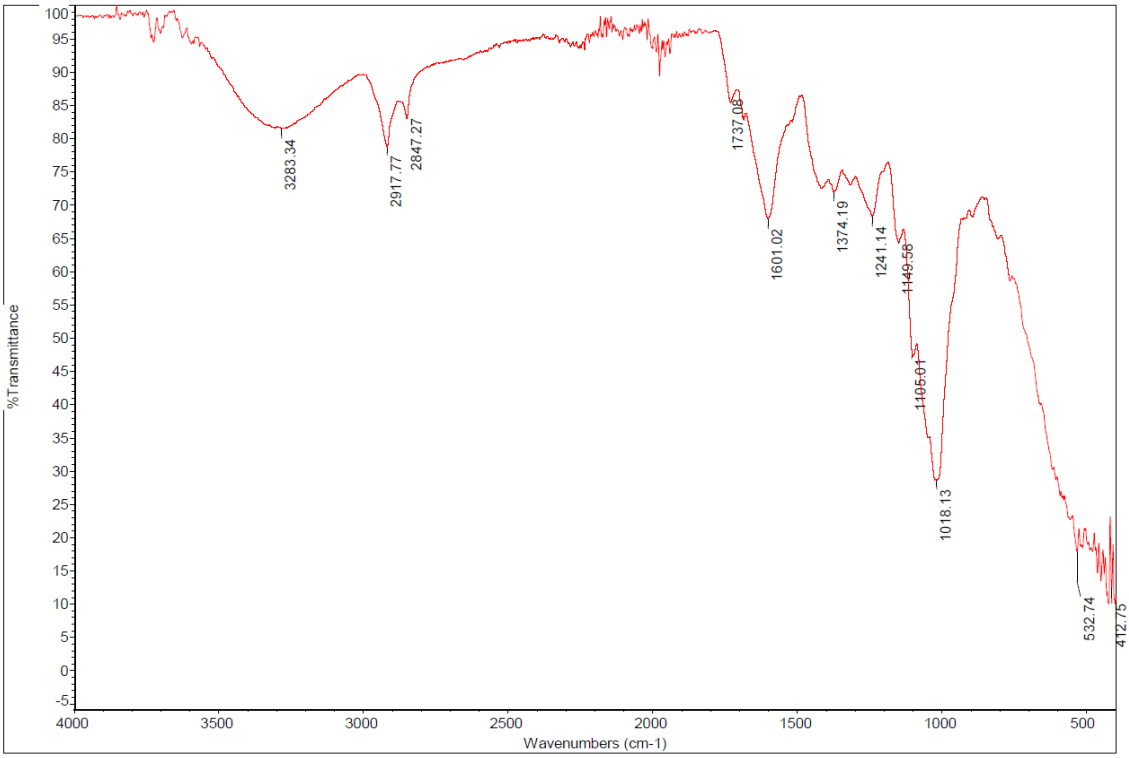
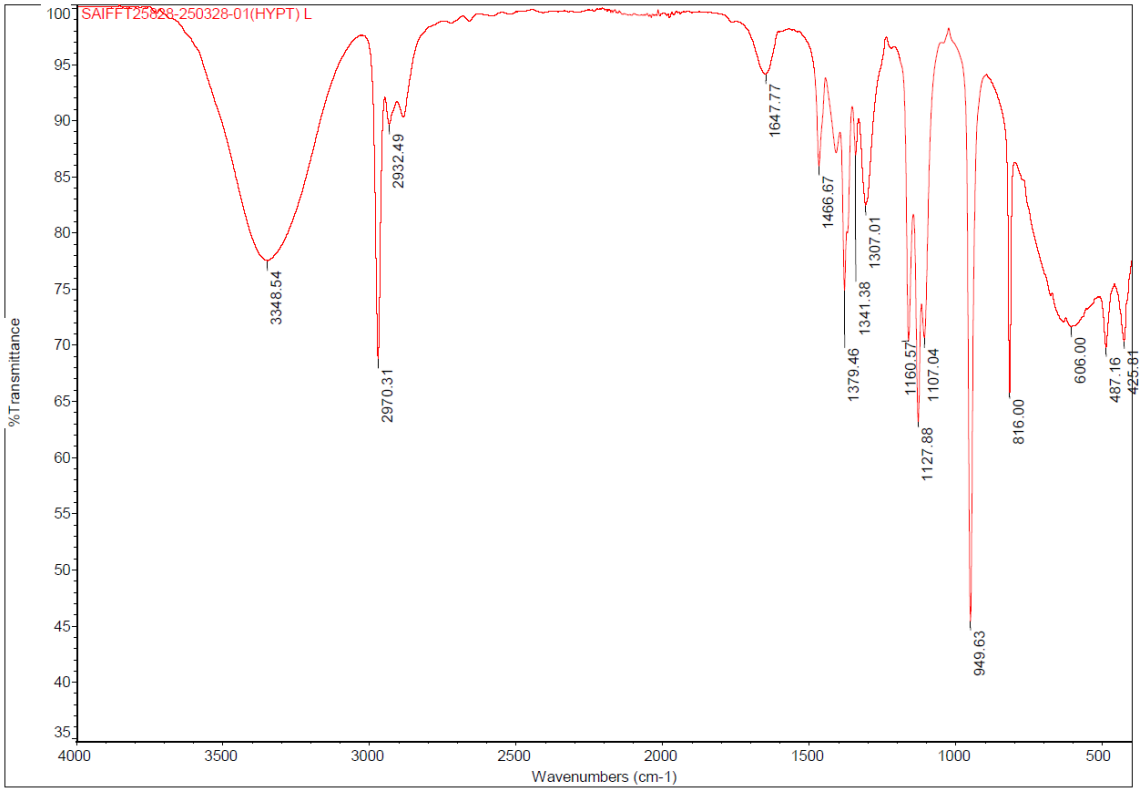


Figure 2: FTIR spectra of Hyptis suaveolens leaf powder



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