SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

Project Report of 2022: SVP-2206

"DNA BARCODING: AN EMERGING TECHNOLOGY FOR MOLECULAR IDENTIFICATION OF MEDICINAL PLANTS"

IQAC

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SRIVIPRA PROJECT 2022

Title: DNA Barcoding: An emerging technology for molecular identification of medicinal plants

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SRIVIPRA-2022

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This is to certify that this project on **"DNA Barcoding: An emerging technology for molecular identification of medicinal plants" (SVC-2206)** was registered under SRIVIPRA and completed under the mentorship of **Dr. Amit Vashishtha, Dr. Aditi Kothari Chhajer and Dr. Neeti Mehla** during the period from 21st June to 7th October 2022.

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Acknowledgements

First and foremost, we would like to thank our Principal Prof. C. Sheela Reddy for giving us an opportunity to work on the topic of DNA barcoding of medicinal plants as a part of the SRIVIPRA Project.

We extend our gratitude to our convenors, Dr. Sharda Pasricha and Dr. S. Krishnakumar, for keeping faith in us.

We take this opportunity to thank Dr. Amit Vashishtha, Dr. Neeti Kothari, and Dr. Neeti Dhaka for being a constant guiding light for us, supporting us and motivating us at each phase.

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Introduction

Medicinal plants are found worldwide and are of immense importance. India is the second-highest producer after China and harbors approximately 7,500 species of medicinal plants (Kala et.al., 2006). The therapeutic value may be attributed to different parts of plants. For example, in the case of *Tinospora cordifolia* (giloe), its leaves are a boon in Dengue as they help in improving platelet count; its stem is helpful during acidity, constipation and in improving digestion. Like *Tinospora*, many plants have diverse medicinal applications. In many countries like India, China, and Korea some herbs have been used since time immemorial. Their knowledge has been passed down through generations by word of mouth and by practice. For instance, turmeric milk is a pretty famous remedy in Indian culture for improving immunity and healing wounds. Recently, it has been found that turmeric is beneficial for cancer patients too (National Cancer Institute, 2022).

Due to their medicinal properties, they are used for treatments in different systems of medicine. In Ayurveda, commonly used plants include Ashwagandha, Boswellia, Amla, etc. (Petre et.al.,2019). In Siddha, plant-originated drugs are known as mooligai moolaporutkal (National health portal of India, 2015). Because of such properties, these plants are of immense importance.

The utility of medicinal plants has seen an enormous growth worldwide. Right from their extract to phytopharmaceuticals, nutraceuticals and cosmeceuticals- medicinal plants have it all. The increased demand calls for huge trade at both the domestic and international levels (Vasisht et. al., 2016). Both export and import of medicinal plants prove to be a boon for the economic growth of the country. China and India are the two major production centres of medicinal plants, having more than 40 percent of the global biodiversity (Singh et. al., 2008). The international market for the trade of medicinal plants is over US$ 60 billion per year and seems to be growing at the rate of 6 percent per annum (Singh et. al., 2008). India is estimated to possess more than 8000 species of medicinal plants and is involved in their trade. For instance, Neem leaves (or even the powdered form) have been used for medicinal purposes since times unknown- for detoxification, as an

immunity booster, a mosquito repellent, for treating wounds and many other uses. Japan, Korea, UAE, Vietnam and Sri Lanka are its major export centres, helping India generate Rs. 86.10 lakh by export of 260.94 tonnes (Singh et. al., 2008).

Apart from China and India, other countries involved in the trade of medicinal plants are Germany, the USA, and Hong Kong.

With the increased demand for medicinal plants worldwide, there is often a demand-supply gap due to which cheap substituent and adulterated products have found their way into the system.

Adulteration of medicinal plants

Adulteration can be defined as the act of worsening the quality of a substance by the addition of another substance. Adulterants are usually sub-standard varieties of crude or inferior drugs or artificially prepared commodities. The unethical practice of adulteration by drug manufacturers would not only reduce the efficacy of the drugs but also affect the trust of the people in the traditional healthcare systems.

But adulteration is not always intentional, it can be accidental too. While the former is usually practiced by traders who are reluctant to pay high prices for the superior quality of original plant materials and thus lean towards the much cheaper but poor-quality substitutes, in-deliberate adulteration occurs without the ill intention of the manufacturer/trader.

The process of adulteration can be carried out by numerous methods: inferiority (adulterant is similar to the crude drug, but has the poorer quality and cheaper cost), spoilage (may occur due to microbial attack), deliberate deterioration, admixing, use of synthetic drugs and artificially synthesized drugs. In India, *Phyllanthus* (Euphorbiaceae) is an important species of medicinal plant traded as a raw herbal drug (Ved and Goraya, 2008; Srirama *et. al.*, 2010). The species is also traded in powder form for the derivation of some phytochemicals for the treatment of liver disorders (Kamble *et. al.*, 2008; Srirama *et. al.*, 2010). Khatoon *et. al.* (2006) studied three species of *Phyllanthus* (*Phyllanthus amarus*, *Phyllanthus fraternus* and *Phyllanthus maderaspatensis*) which are frequently mixed due to high morphological similarity, leading to a change in the original phytochemistry. But only *Phyllanthus amarus* was found to contain phyllanthin and hypophyllanthin, the two compounds believed to be responsible for the hepato-protective activity (Calixto *et. al.*, 1998; Srirama *et. al.*, 2010).

Cheaper cost and profit are not the sole reasons behind adulteration. Others include confusion in vernacular names, lack of knowledge about authentic sources, lack of authentic plants, unscientific collection, and the high price of the drug in the market, with the intention of enhancing profits.
The adulterant may be closely related to the original species but still has the potential to cause damage to human health. Hence, it is vital to prevent adulteration, right from the collection of the plant material to the sale of the final product.
Due to advancements in every field of science, adulteration can be evaluated by the following methods: Morphological or organoleptic tests, microscopic evaluation, chemical evaluation, physical evaluation, chromatography, spectrophotometry, radioimmunoassay, biological evaluation (Sreelekshmi et. al., 2017).

NAME OF PLANT	**ADULTERANT**	**BIOCHEMICAL TEST FOR GENUINITY**
1. *Crocus sativus* (Kumkuma)	Stamen of Safflower and Marigold	Stigma will turn from blue to purplish- red when sprinkled with conc. H_2SO_4
2. *Curcuma longa* (Haridra)		A deep crimson colour is seen when the powdered drug reacts with conc. H_2SO_4 or a mixture of conc. H_2SO_4 and alcohol
3. *Cinnamomum camphora* (Karpoora)	Gum, alum, starch, gum	Original sample will float, burn quickly and dilutes instantly when put in chloroform/solvent ether
4. *Commiphora mukul* (Guggulu)	Shallaki secretions, sand, wood and bark pieces	When particles are put in water, they become round. When put on fire, they liquify and give white fumes. Sreelekshmi *et. al.*,2017

However, these biochemical tests can be ambiguous. Any external property of a biological sample can vary with time. Consequently, we require a more stable property to compare and identify them. DNA serves this purpose well as it is a stable macro molecule found in all tissues.

DNA Barcoding

In order to authenticate the purity of food materials, numerous technologies have been put into use such as spectrophotometer, HPLC, DNA barcoding, GC-MS, etc. Amongst these techniques, DNA barcoding provides a biotechnological advantage involving the use of short standardized sequences of DNA about 400-800 bp long from mitochondrial (eg-COI) or plastidial (eg-rbcl) of nuclear origin (eg-ITS) to help analyze as well as classify the source of various food commodities, (Nehal *et. al*, 2021).

DNA barcoding can be defined as a taxonomic procedure in which a small region of DNA either from nuclear or organelle genomes is used as a genetic marker to identify any biological specimen on earth. (Hebert et al. 2003).The term 'DNA barcode' as a taxon identifier was first proposed by Paul Hebert of the University of Guelph in 2003. He recommended that the 5′ end of cytochrome c oxidase 1 (CO1) from the mitochondrial genome was sufficient to generate DNA barcodes for the identification of animals.(Techen *et. al*, 2014).

The primary motive of this novel technique is to create a community resource database comprising a variety of DNA sequences that can be easily accessed for quick identification, distinction, and taxonomic classification of organisms on earth. Genetic markers derived from a small region of a gene are often used to distinguish species, varieties, and even inter-varieties based on their particular characteristics. (Bandopadhaya Shruti *et al.*, 2013).

DNA barcodes can be compared to industrial barcodes which act as unique identifying tags for a wide variety of commercial products. Small gene segments used as genetic markers comprise information unique to every species. Therefore, the process of DNA barcoding has become a worldwide standard for the identification of all plants, animals, and even fungi. It is known that a small gene region from the mitochondrial CO1 gene acting as a genetic marker is used for the identification of animal species on earth such as fish, flies, birds, etc. While for the identification of land plants a combination of two genes from chloroplast regions is used, they are "matK" and "rbcl". Amongst the protein-coding regions of mitochondrial genes, the COI barcoding region has been given higher preference as the core molecular diagnostic system for the apt identification of animal species. (Hebert *et al.*, 2003; Luo *et al.*, 2011).

DNA Barcoding in Medicinal Plants

In the mid of growing modernity, the 21st century has seen an increased demand for herbal medications over chemically synthesized medicines with higher side effects. Hence DNA barcoding as a scientific tool is emerging to be a pioneer for medical/pharmaceutical research in providing a common database arena to find genetic information of any herbal the medicinal plant, which could be used in the making of herbal medicines.

Such a database could provide complete information on medicinal plants based on their genetic barcode stored in the database. To obtain such barcodes for every plant species, numerous methods have been devised to create genetic markers from the DNA of every plant so that clear and authentic information regarding the chemical effects (positive/negative) of the plant can be derived before it is used for medicinal purposes.DNA is a stable macromolecule as compared to RNA and is not affected by external cues and is present in all tissues. Hence, the development of DNA-based genetic markers has gained momentum for the authentic identification of medicinal plants using DNA barcoding (Techen *et. al,* 2014).

The Consortium for the Barcode of Life Plant Working Group (CBOL) has evaluated seven genomic regions on chloroplast all across the plant kingdom and proposed a combination of matK and rbcL as plant barcodes. A high level of universality but less species resolution is provided by rbcL whereas matK gives high resolution but less universality, (Combik Mirek, 2015).

A combination of the above two can help to achieve maximum species distinction. Nevertheless, in closely related species, the differentiating ability of these two markers is low. Hence, the China Plant BOL Group proposes plant barcodes in order to achieve maximum identification rates even in closely related species. (Natascha Techen, 2014).

Thus, DNA barcoding has played a crucial role in the classification of medicinal plants, the identification of substitutes/adulterants, and the regulation of the pharmaceutical market. The present technical means can effectively identify and utilize most of the plant raw materials and rough-wrought products towards the enhancement of the medicinal market sector. (Yu *et.al,* 2021).

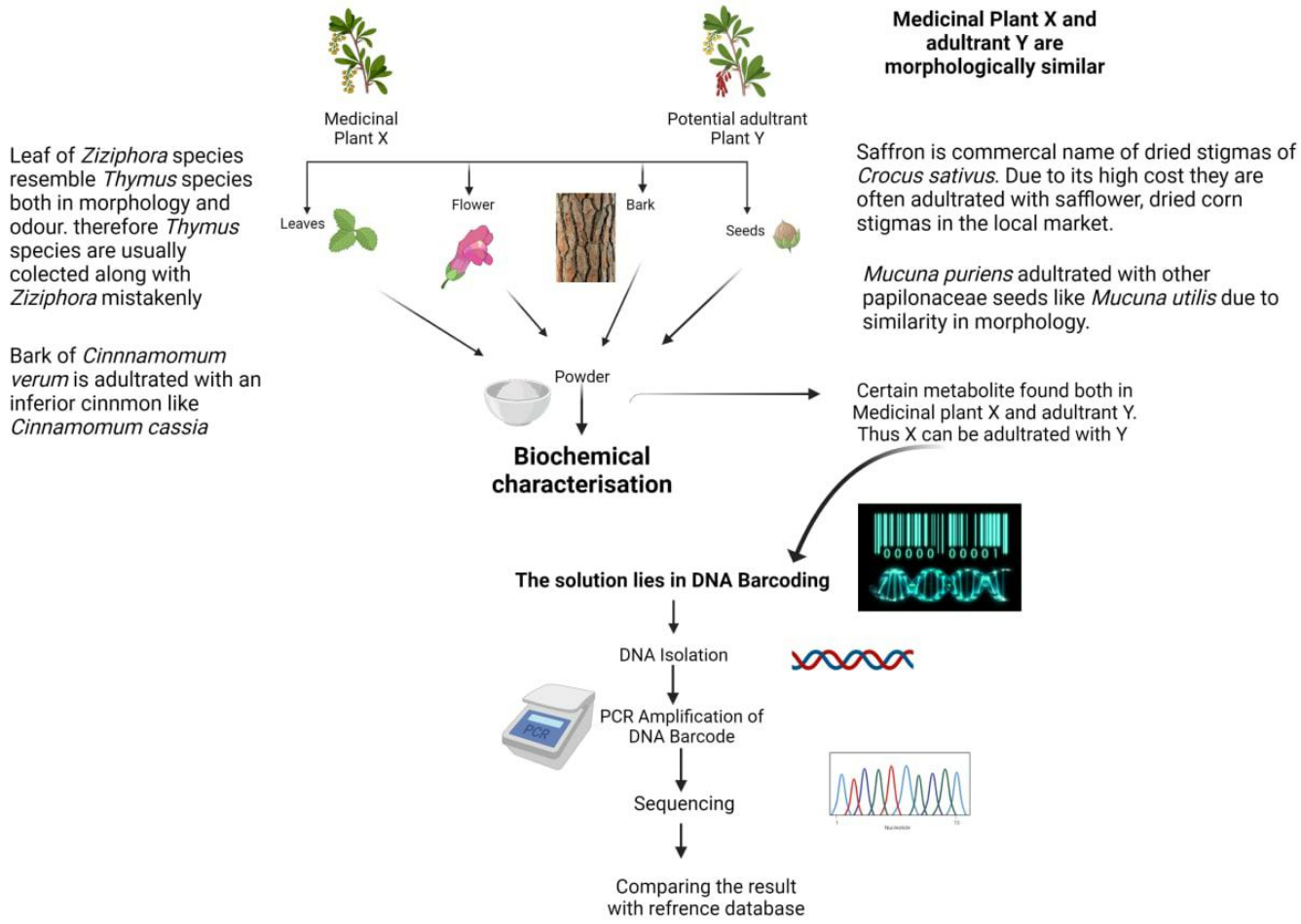

Figure: Distinguishing a medicinal plant with a potential adultrant.

Table: Loci used for DNA Barcoding in Medicinal Plants

Sno	Plant name	ITS	ITS1	ITS2	matK	rbcL	rpoC	rpoB	trnH-pbsA	trnl-trnF1	trnl-trnF2	Reference
1	Medicinal Orchids	0	0	1	1	1	0	0	1	0	0	Raskoti and Ale (2021)
2	*Aquilaria sinensis*	0	0	1	1	0	0	0	1	1	1	Kang *et.al* (2021)
3	*Aquilaria yunnanensis*	0	0	1	1	0	0	0	1	1	1	Kang *et.al* (2021)
4	*Angelica spp.*	1	0	1	1	1	0	0	1	0	0	Techen *et.al*
5	*Thunbergia laurifolia*	0	0	0	1	1	1	0	1	0	0	Singtonat *et.al* (2015)
6	*Boerhavia diffusa*	1	0	1	0	0	0	0	1	1	1	Selvaraj *et.al* (2012)
7	*Galphimia glauca*	0	0	0	1	1	1	0	1	0	0	Borroto et.al (2019)
8	*Mitragyna speciosa*	1	0	0	1	1	0	0	1	0	0	Tungphatthong et.al
9	*Aristolochia tangala*	0	0	1	1	1	0	0	1	0	0	Dechbumroong et.al (2018)
10	*Meconopsis viguier*	1	1	1	0	0	0	0	0	0	0	Rong Li and Zhiling Dao (2011)
11	*Ochradenus arabicus*	1	0	0	0	1	1	1	0	0	0	Khan et.al (2012)
12	*Lonicera japonica*	1	0	1	1	1	0	0	1	1	1	Sun Zhiying et.al (2010)
13	*Paris polyphylla*	1	0	0	0	0	0	0	0	0	1	Tao Liu and Yunheng Ji (2011)
14	*Panax ginseng*	1	0	0	1	1	1	1	1	0	0	Zuo Y. et al 2010

15	*Panax quinquefolius*	1	0	0	1	1	1	1	1	0	0	Zuo Y. et al 2010
16	*Panax notoginseng*	1	0	0	1	1	1	1	1	0	0	Zuo Y. et al 2010
17	*Smilax calophylla*	0	0	0	0	1	0	0	0	0	0	Cahyaningsih et.al (2022)
18	*Etlingera solaris*	0	0	1	0	1	0	0	1	0	0	Cahyaningsih et.al (2022)
19	*Rhododendron macgregoriae*	0	0	1	1	1	0	0	1	0	0	Cahyaningsih et.al (2022)
20	*Justicia gendarussa*	0	0	1	1	1	0	0	1	0	0	Cahyaningsih et.al (2022)
21	*Oberonia lycopodioides*	0	0	1	1	0	0	0	1	0	0	Cahyaningsih et.al (2022)
22	*Justicia gendarussa*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
23	*Saturogyne elongata*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
24	*Pangium edule*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
25	*Spondias malayana*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
26	*Toxicodendndron succedanem*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
27	*Ancistrocladus tectorius*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
28	*Anaxagorea javanica*	0	0	0	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
29	*Dasymaschalon dasymaschalum*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)

30	*Alstonia macrophylla*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
31	*Alstonia scholaris*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
32	*Alyxia reinwardtii*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
33	*Alyxia reinwardtii*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
34	*Hoya diversifolia*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
35	*Rauvolfia sepentia*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
36	*Trevesia burckii*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
37	*Cibotium barometz*	0	0	1	0	1	0	0	0	0	0	Cahyaningsih et.al (2022)
38	*Decalobanthus mammosus*	0	0	0	0	1	0	0	0	0	0	Cahyaningsih et.al (2022)
39	*Erycibe malaccensis*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
40	*Rhohododendron macgregoriae*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
41	*Acalypha grandis*	0	0	1	0	1	0	0	0	1	0	Cahyaningsih et.al (2022)
42	*Smilax zeylanica*	0	0	1	0		0	0	0	0	0	Cahyaningsih et.al (2022)
43	*Parkia timoriana*	0	0	0	0	1	0	0	0	0	0	Cahyaningsih et.al (2022)

44	*Pangium edule*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
45	*Nervilia plicata*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
46	*Nervilia concolor*	0	0	1	0	1	0	0	0	0	0	Cahyaningsih et.al (2022)
47	*Benstonea affinis*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
48	*Cibotium barometz*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
49	*Dasymaschalon dasymaschalum*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
50	*Galearia filiformis*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
51	*Grammatophyllum speciosum*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
52	*Aglaonema commutatum*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
53	*Ventilago madraspatana*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
54	*Phanera fulva*	0	0	0	0	1	0	0	0	0	0	Cahyaningsih et.al (2022)
55	*Premna serratifolia*	0	0	0	0	1	0	0	0	1	0	Cahyaningsih et.al (2022)
56	*Anaxagorea javanica*	0	0	0	1	1	0	0	0	0	0	Cahyaningsih et.al (2022)
57	*Aquilaria hirta*	0	0	1	1		0	0	0	1	0	Cahyaningsih et.al (2022)

58	*Rhododendron macgregoriae*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
59	*Aegle marmelos*	1	0	0	1	1	0	0	1	0	0	Cahyaningsih et.al (2022)
60	*Coriandrum sativum L*	1	0	0	1	1	0	0	1	0	0	Cahyaningsih et.al (2022)
61	*Morinda citrifolia*	1	0	0	1	1	0	0	1	0	0	Cahyaningsih et.al (2022)
62	*Ficus deltoidea*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
63	*Galearia filiformis*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
64	*Kadsura scandens*	0	0	1	1	0	0	0	0	1	0	Cahyaningsih et.al (2022)
65	*Lunasia amara*	0	0	1	0	0	0	0	0	0	0	Cahyaningsih et.al (2022)
66	*Nepenthes gracilis*	0	0	0	1	0	0	0	0	0	0	Cahyaningsih et.al (2022)
67	*Nepenthes reinwardtiana*	0	0	1	1	0	0	0	0	0	0	Cahyaningsih et.al (2022)
68	*Nervilia plicata*	0	0	0	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)
69	*Pangium edule*	0	0	0	1	1	0	0	0	0	0	Cahyaningsih et.al (2022)
70	*Rauvolfia serpentina*	0	0	1	1	1	0	0	0	1	0	Cahyaningsih et.al (2022)

Future scope of DNA barcoding in India

On account of the increasing demand for herbal products and medicines in India as well as worldwide, it has now become extremely important to check for authentication of the ingredients used in the products. Not only are the substituents and adulterants easily accessible and easy to replace the original species of medicinal plants but also they may cause serious health problems if consumed wrongly. Thus, DNA barcoding, which enables the identification and classification of specific plant species is highly necessary. DNA barcoding can serve as a process to keep a check on the raw material (parts of the plant such as roots, seeds, flowers, etc.) in their dried form to be precisely identified by the industries which aim to produce toxin-free herbal products. DNA barcoding is an emerging technology that seeks its demand among medicinal plants. Assigning definite DNA barcodes to specific medicinal plant species is a very efficient way of checking new samples for their authenticity and to check adulteration.

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