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Iranian traditional medicine for treatment of type II diabetes, anxiety and hypertension with introduction of zebrafish model system for their screening

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Abstract
The use of medicinal plants for treatment of Type II diabetes, hypertension and anxiety is very common among non-industrialized colonies because these remedies are easily available and low cost than novel pharmaceuticals. Medicinal plants are important sources of new drug candidates. Preclinical assessments of drug toxicity and efficacy rely largely on mice as the animal model of choice, but zebrafish is increasingly used for early preclinical stages in the research pipeline due to number of compelling inherent advantages including: rapid organogenesis, high fecundity, optical translucency, year-round breeding, and availability of genetic tools. The aim of this paper is to review the literature on a panel of selected Iranian medicinal plant extracts. I focus on a selection of medicinal plants traditionally used in Iran to treat type II diabetes (13species), hypertension (12species) and anxiety (12 species). Next, I review the literature for evidence of active part of these plants. At the end, I will discuss the zebrafish assays that will be used for screening these plants for anti-diabetic, antihypertensive and anxiolytic effects. I hope that this communication will be a useful starting point to consider the value of ethnobotanical drug discovery approaches combined with the zebrafish embryo alternative screening assays in future studies.

Keywords: Iranian medicinal plants, Diabetes, Anxiety, Hypertension, Zebrafish embryo

1. Introduction
Medicinal plants utilized in Iranian Traditional Medicine (ITM) have had, and still play, a significant role in the avoidance and treatment of illnesses. Less effectiveness of modern medicine has given a place to the traditional remedies to exhibit themselves as an alternative medicine [1]
Iran covers 1.64 million km^2 and has 7,500-8,000 native plant species of flowering plants. Iran is an ancient country in utilization of medicinal plants for treatment and prevention of illness. Because of abundant diversity of climate and geographical conditions of Iran, many different medicinal species are cultivated there. [2] Different plant parts including leaves, roots, flowers and seeds are used for the production of drugs. In addition, oils and other extracts of various plant species constitute very potent natural biologically active agents. [3, 4]
Herbal products are the whole plant or parts of it or its crude or processed products that are used for prophylactic treatment and mental and physical diseases. [2] In general, herbal products are classified into three different divisions or kinds.
The first are medicinal plants including crude plant materials including seeds, stems, wood, bark, root, flower, leaves and other plant parts, which may be undivided as a whole, fragmented or fractioned or in the form of powder. These are packaged for trade at markets in the form of herbal teas (bags) or sachets.
The second division comprises herbal preparations. These are the basis for last staged herbal products (drugs, hygienic and cosmetic products) and contain extracts and essential oils. Herbal preparations are produced by fractionation, extraction, concentration, purification or other physical processing of raw plant extracts. The third division comprises compound herbal preparations made from one or more herbs and possibly contains excipients (inactive carriers) in addition to active ingredients. Herbal drugs, to which chemically defined active substances (such as synthetic compounds and/or isolated constituents from herbal materials) have been added, are not considered to be herbal [5].

1.1 The need for alternatives to existing chemical drugs and ineffectiveness of current screening methods
Ineffectiveness of some existing chemical drugs, their sometimes serious side effects (including chronic toxicity), leads to use of herbal medicine as a form of complementary

and alternative medicine [6]. This is for instance is the case with drugs taken against epilepsy, a brain disorder that affects about 50 million people worldwide.[7]Anti-epileptic drugs have many possible side-effects. Common side-effects that affect people in later life include unsteadiness and drowsiness. Other anti-epileptic drugs such as, sodium valproate, phenytoin phenobarbital and carbamazepine may lead to reduction in bone density. Alternative medicines for treatment of epilepsy might help to reduce these side effects [7]. Table 1 summarizes some of the Iranian that have been screened for Anticonvulsant effects .As can be seen ,antiepileptic compounds have been characterized from *Ferula, Nigella sativa* (Thymoquinone) and *Piper longum*(piperine) [8-10]. By contrast Shojaii [7] has pointed out that other antileptic plants in TIM have not been sufficiently validated with modern research and *Pistacia lentiscus* gum (Mastaki), *Bryonia alba* (Fashra), *Ferula persica* (Sakbinaj), *Ecballium elaterium* (Ghesa-al Hemar), and *Alpinia officinarum* (Kholanjan) as examples [7].

Table 1: Anti-epileptic effects of traditionally used medicinal plants in Iran

Plant species	Family name	Parts used in TIM	Animal model	Preparation/extract tested in animal model	References for animal study
Terminalia chebula	Combretaceae	Fruits	PTZ, MES, and PC* PTZ, ICES*	Ethanolic extract of fruits Chloroform fraction of root	[17]
Anacyclus pyrethrum	Asteraceae	Roots	MES PTZ,MES	Root ethanolic extract Hydroalcoholic root extract	[7-9]
Pimpinella anisum	Apiaceae	Seed	PTZ, MES PC	Aniseseed essential oil Methanol seed extract	[10-12]
Laurus nobilis	Lauraceae	Seed	PTZ, MES	Leaf essential oil	[18]
Origanum majorana	Lamiaceae	Aerial parts	PTZ, MES PTZ	Extracts with petroleum ether, chloroform, acetone, methanol, water Aqueous seed extract	[19]
Nigella sativa ***	Ranunculaceae	Seed	PTZ PTZ,MES PTZ, strychnine, and 4-aminopyridine	Seed essential oil Thymoquinone Aqueous fruit extract Piperine	[14, 20, 21] [15, 16, 22, 23] [24]
Piper longum	Piperaceae	Fruits	Pilocarpine Threshold doses of kainite L-glutamate, N-methyl-D-aspartate guanidinosuccinate		[25, 26]
Ferula gummosa	Apiaceae	Gum resin	PTZ, MES	Fruit essential oil seed acetone extract	[27-29]
Ferula assa-foetida	Apiaceae	Gum resin	PTZ, MES	Gum ethanolic extract	[30, 31]
Brassica nigra	Brassicaceae	Fruits	PTZ	Seed extract	[30, 31]
Caesalpinia bonducella	Fabaceae-Caesalpiniaceae	Fruits	PTZ, MES, strychnine, and PC	Seed pet ether extract	[32]
Lavandula stoechas	Lamiaceae	Arial parts	PTZ	Aqueous-methanolic extract of flower	[33]
Ruta graveolens	Rutaceae	Arial parts	PTZ	Hydroalcoholic extract	[34]

*PTZ: pentylenetetrazole, MES: maximum electroshock, PC: picrotoxin, ICES: increasing current electroshock.
**Effect of piperin on oral bioavailability of phenytoin

1.2 Alternative medicine for anxiety, type II diabetes and hypertension

Diabetes, hypertension and anxiety disorders are serious medical threats of public health. [35-37]. There is a strong need for new drugs for treatment and prevention of these diseases. Traditionally-used medicinal plants are an undervalued potential source of alternative treatments. The aim of this review is to gather scientific evidence of effectiveness of natural constituents of plants that are used medicinally in Iran for treatment of these diabetes, heart disease and anxiety disorders.

Based on a literature study, I have selected species that are traditionally used in Iran with remedial properties for treatment of hypertension, diabetes and anxiety (Tables 2-4) these will be discussed in further detail below.

1.3 Anti-diabetic plants

Anti-diabetic effects of some of the medicinal plants mentioned in TIM like *Haussknechtia elymaitic, Achillea wilhelmsii, Cichorium intybus* were studied by Iranian scientists [38]. From some of these plants, such as *Urtica dioica, Spiranthes spiralis* [39, 40], the active constituent responsible for anti-diabetic effect was identified and studied. For some of the medicinal plants used in TIM such as *Gymnadenia* [39] *Haussknechtia elymaitica* [38], insufficient studies have yet been carried out to confirm their effectiveness in treating and/or preventing diabetes. Screening extracts of these plant species in zebrafish embryo is feasible to obtain more knowledge about their effects as zebrafish emrbryo can be used as an alternative animal model for better evaluation of the plant's extracts [56].

Table 2: Selection of Iranian medicinal plants used to treat diabetes

Scientific name	Family	Persian vernacular name	English vernacular name	Plant Part used	References
Haussknechtia elymaitica	Apiaceae	Kelos-e kuhi	White celery	Aerial part	[38]
Achillea wilhelmsii	Asteraceae	Berenjas	Yarrow	Flower	[38]
Cichorium intybus	Asteraceae	Kashni	Chicory	Leaves, stems and flowers	[38]
Tanacetum polycephalum	Asteraceae	Mokhaleseh	Feverfew	Leaves, flowers	[41]
Berberis integerrima	Berberidaceae	Zereshk	Common berberry	Flower, hypoderm of stems, epiderm of roots	[38]
Citrullus colocynthis	Cucurbitaceae	Gojey-e Abu; jahl,khiar Hendavaneye sangi	Colocynth bitter cucumber, bitter apple	Seeds	[42]
Trigonella foenum-graecum	Fabaceae	Shanbalileh	Fenugreek	Aerial part, seed	[43]
Satureja khuzistanica = Teucrium polium	Lamiaceae	Marzeh khuzestani; Chez Koohi; Kalporeh	Cat thyme, hulwort, mountain germander	Aerial parts, leaves, flowers	[44]
Dactylorhiza iberica	Orchidaceae	Salabe Khaldar	Marsh orchid	Tubers	[40]
Gymnadenia conopsea	Orchidaceae	Salabe moatar	Fragrant orchid	Tubers	[40]
Platanthera bifolia	Orchidaceae	Salabe parvaneye saghir	Lesser butterfly-orchid	Tubers	[40]
Spiranthes spiralis	Orchidaceae	Salabe giso bano	Lady's tresses orchid	Tubers	[40]
Urtica dioica	Urticaceae	Gazaneh; Gazgazuk	Stinging nettle	Aerial parts, leave and stems	[39]

1.4 Anti-anxiety plants

Anti-anxiety effects of some of the medicinal plants mentioned in TIM like *Nepeta persica Salvia reuterana,* were studied by Iranian scientists From some of these plants, such as *Valeriana officinalis, Citrus aurantium, Nepeta persica* the active constituent responsible for anti-anxiety effects was identified, isolated and studied. For some of the medicinal plants used in TIM such as like *Nepeta persica* and *Salvia reuterana* insufficient studies have yet been carried out to confirm their effectiveness in treating and/or preventing anxiety. Zebrafish embryo can be modeled for better evaluation of plant species in their effect and property [53].

Table 3: Selection of Iranian medicinal plants traditionally used to treat anxiety.

Scientific name	Family	Persian vernacular name	English vernacular name	Plant Part used	References
Echium amoenum	Boraginaceae	gol-e gavzaban	Red feathers	Flowers	[45]
Crocus sativus	Iridaceae	Zaferan	Saffron	Aerial parts	[42]
Nepeta persica	Lamiaceae	Pune farsi	Persian catmint	Aerial parts	[45]
Salvia reuterana	Lamiaceae	Maryam Goli		Aerial parts	[45]
Stachys Lavandulifolia	Lamiaceae	Chay-e-kohi	Betony	Aerial parts	[45]
Cinnamomum zeylanicum	Lauraceae	Darchin	Cinnamon	Infusion of the leaves	[46]
Gymnadenia conopsea	Orchidaceae	Salabe khushboo	Fragrant orchid	Leaves, roots	[47]
Glaucium spp.	Papaveraceae	Shaghayegh	Horn poppy	Infusion of the leaves	[46]
Citrus aurantium	Rutaceae	Narang	White round or blond oranges	Leaves and flower	[48]
Salix aegyptiaca	Salicaceae	Bidmeshk	Musk willow	Petals	[49]
Coriandrum sativum	Apiaceae	Geshniz	Coriander	Aerial part Fruit	[50]
Valeriana officinalis	Valerianaceae	Sonbol-ot-Teyb	Indian valerian	Infusion of the leaves	[46]

1.5 Antihypertensive plants

Anti-hypertensive effects of some of the medicinal plants mentioned in TIM like *Vaccinium arctostaphylos and Melilotus officinalis were* studied by Iranian scientists From some of these plants, such as the active constituent responsible for anti-hypertensive effects was identified, isolated and studied. For some of the medicinal plants used in TIM such as *Crataegus* spp and *Vaccinium arctostaphylos*. Insufficient studies have yet been carried out to confirm their effectiveness in treating and/or preventing hypertension. It is feasible to model zebrafish embryo for study of hypertension and screen antihypertensive plants in zebrafish as it meets possible criteria as a model for the disease. [58]

Table 4: Selection of Iranian medicinal plants traditionally used to treat hypertension

Scientific name	Family	Persian vernacular name	English vernacular name	Plant parts used	References
Allium hirtifolium	Alliaceae	Mousir	Persian shallot	Bulbs	[41]
Haussknechtia elymaitica	Apiaceae	Kelos-e kuhi	White celery	Aerial parts	[38]
Achillea wilhelmsii	Asteraceae	Berenjas	Yarrow	Flowers	[51]
Citrullus colocynthis	Cucurbitaceae	Gorjey-e Abujahl.Khiar gorgu,Hendevaneye sangi	Colocynth bitter cucumber, bitter apple	Seeds	[38]
Vaccinium arctostaphylos	Ericaceae	Siah Gileh	Caucasian whortleberry	Fruits	[52]
Melilotus officinalis	Fabaceae	Aklilolmolk	Yellow sweet clover, yellow melilot, ribbed melilot	Leaves	[41]
Fumaria parviflora	Fumariaceae	Shah-Tareh	Fine leaf fumitory, Indian fumitory	Aerial parts	[38]
Ajuga austroiranica	Lamiaceae	Alaf-e Titarei		Aerial parts	[38]
Orchis spp.	Orchidaceae	Salabe Mashregh	Levant Salap	Leaves, tubers	[17]
Ziziphus nummularia	Rhamnaceae	Ramalik	Indian jujube	Fruits	[38]
Crataegus spp.	Rosaceae	Siseh	Hawthorn	Leaves, fruits, flowers	[38]
Urtica dioica	Urticaceae	Rishe-e gazane	Nettle root	Aerial Parts	[38]

1.6 Zebrafish embryo as an emerging animal model for screening of plant's extracts

The zebrafish (Danio rerio) is an emerging model organism for drug discovery for human diseases. [53]Its positive features as a research model include similarities with mammals in key physiological pathways, functional domains of key disease gene, high rate of fecundity, external fertilization (allowing embryos to be exposed to drug)rapid development, optical transparency of embryos and availability of genetic tools.[53]A particular advantage for screening natural products, such as herbal medicines, is that relatively small quantities of the test material are consumed .This is because ,in a 96 –well plate, each embryo is maintained in only 250µL of buffer [56] Disadvantages of zebrafish embryo model include the presence of a relatively impermeable extra –embryonic membrane (chorion) at early stages [56,58] and the lack of information about ADME (absorption, distribution, metabolism and elimination) of drugs in zebrafish. Also, since the compounds to be screened have to be added to the aqueous buffer in which the embryos are swimming, screens are more-or-less restricted to water soluble compounds.

In this paper, I have proposed the zebrafish embryo as model for anxiety because it shows conserved stress responses.[53] Thigmotaxis (or wall hugging) is a response to stressful stimuli in mammals and has shown to be present also in zebrafish larvae.[53]Furthermore, anxiety like behavior , it is attenuated or ameliorated by the same pharmacological agents in human and zebrafish.[53] Zebrafish apparatus used for study of anxiety and compound screening in which The zebrafish larvae are exposed to a light-dark cycle; the darkness is stressful to the fish and their swimming pattern is recorded by video and analyzed automatically. Anxiolytic compounds have been shown to suppress the dark-induced stress-response, while anxiogenic compounds enhance it.[53].Zebrafish larvae can be used to screen antidiabetics and antihypertensive. The zebrafish can be used to model some aspects of type II diabetes.[56] The zebrafish larvae is an excellent model ;during fasting ,it shows expression of the pck1 gene having key role in the regulation of gluconeogenesis , as a human biomarker for type II diabetes. Thus zebrafish's fasting stage is an excellent marker for drug screening for diabetes and availability of gene expression analysis of pck1 gene, human's marker gene for diagnosis of diabetes makes zebrafish larvae as an excellent animal model system for discovery of anti-diabetic drugs[56].For antihypertensive drugs at the genetic level , it is possible to examine the expression of endothelial Nitric oxide Synthase gene for introduction of it as a potential biomarker for medicinal plant screening in hypertension treatment. The principle behind of this mechanism is that some of selected medicinal plants like *Citrullus colocynthis* contain larginine used as substrate which oxidized by functional eNOS to form L-cirulline which in turn produces Nitric Oxide which is responsible for vasodilatation. By this plan it is possible to model zebrafish for study of cardiovascular diseases and drug screening for hypertension. Zebrafish larvae is chosen for study of genetic related diseases such as diabetes and hypertension and drug discovery for the mentioned disorders due to its meet to the need of facilities for diagnosis of target

markers and similar response of the body ‘s organ to the diseases [58].

2. Conclusions

Herbal remedies are still widely used in world especially in ancient countries like Iran. Ineffectiveness and appearance of reverse side effects of chemical medicines have led to substitute an alternative medicine, natural products are promise candidates for treatment of different diseases like anxiety, hypertension and type II diabetes as they are low cost and harmless. Although some of the medicinal plants used have been researched using modern methods, other plants remain unscreened. The embryos of zebrafish are used as model to measure the efficacy of substances**.** I proposed the use of zebrafish embryo assays a screening model suitable to test the pharmacological properties of traditionally used medicinal plants. The focus on molecular and phenotypic readouts in zebrafish embryo's assays for drugs used in the treatment of anxiety, type II diabetes and hypertension is another aspect of the paper. Not only will this study provide a new insight into Iranian Traditional Medicines and need of traditional medicine as an alternative method of treatment, but it will also help to validate the zebrafish embryo as a suitable model for natural product screening.

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4. Conflicts of Interest

The author declares no conflict of interest.

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