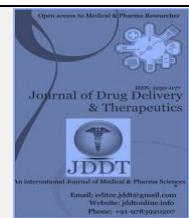


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Review Article

TIRYAQ E ARBA: A classical Unani Formulation to boost immunity

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ABSTRACT

Unani System of medicine is one among the oldest systems of medicine that prevails till date with its efficient drugs derived from animal, plant and mineral resources. Over 2400 years ago the father of medicine, Hippocrates practiced it, however His medicine included a great deal of ancient Egyptian Medicine as well as important components of the ancient Mesopotamian traditions. This system of medicine has a detailed description of drugs that are utilized in many infectious diseases like influenza, pneumonia and other respiratory disorders. Unani scholars have prescribed several single drugs as well as compound formulations for the prevention and treatment of infectious diseases in general. *Tiryaq e Arba* is one such formulation, which is known to improve host immunity anytime or during the outbreak of epidemics, endemics and pandemics. Through this paper, an attempt has been made to present Unani concept of infectious and epidemic diseases and details of *Tiryaq e Arba* with a possible approach to manage Covid-19.

Keywords: *Tiryaq e Arba*, ingredients, epidemics, Unani Medicine.

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Introduction

Unani system of medicine has its roots in ancient Greece, in the teachings of Hippocrates (460–377 BCE). This system flourished to its peak during medieval ages (500–1500 CE) in the Muslim world, mostly in the Arabian Peninsula, Persia, Egypt, Syria, ancient Mesopotamia, etc. In India, it is integrated into the national healthcare system and officially named as Unani system of medicine¹. Unani medicine is based on the Hippocratic concepts of *mizaj* (temperament) and *akhlat* (humours)². Famous scholars of Unani medicine include *Ibn Sina* (Avicenna, 980–1035 CE), *Zakariya Razi* (Rhazes, 865–925 CE), *Ibn Rushd* (Averroes, 1126–1198 CE), and many more³.

Concept of infectious diseases and “waba” (epidemics) in Unani Medicine

A comprehensive literature search indicated that the term ‘*waba*’ is used in Unani literature to describe epidemics and pandemics collectively for diseases which spread in a large geographical area⁴. Epidemics supposed to occur when *ajsam-i-khabitha* (contagion), find a place in air and water. Unani medicine claims that the spread of epidemics occurs through the air, water, and soil or altogether. In relation to

this, Unani physicians promoted preventive measures such as personal hygiene, abstinence from travel, and isolation of the sick to check the spread of disease.

Zakariya Razi (865–925 CE) stated in his book *Kitab al-Mansoori* that most epidemics occur during the autumn season, mostly when previous summer season was humid, and the wind is still⁵, Razi in the 15th volume of his treatise *Kitab al-Hawi* (The Comprehensive Book of Medicine), also stated that change of temperature makes people more susceptible to respiratory infections and stressed this fact and stated that ‘there will always be something common in patients of epidemics, whether a place, food, drink or travel history’⁶. Furthering the view, *Ibn Sina* (980–1035 CE) stated that epidemics spread from one person to another, and one city to another (Sina, 1878). During the 14th-century plague pandemic, Arabian scholar *Ibn Khatib* (1313–1374 CE) stressed that ‘most of the people who come in contact with a plague victim will die’. In the same context, he states, ‘the disease spreads through clothes, utensils and jewelry’^{7,8}. In the same vein, this statement stresses on social distancing and isolation, two important aspects of prevention during COVID 19 pandemic. The 13th-century Persian scholar *Najeebuddin Samarkandi* (d. 1222 CE) mentioned about a type of

epidemic influenza in his treatise *Al-asbab wa-Alamat* (the book of causes and symptoms). In the translated version of book, published by the name of *Sharah Asbab*, the disease is mentioned by the name of *Nazla-e-Wabaiya* (epidemic influenza) characterized by fever, sneezing, sore throat, nasal irritation and malaise and may also suffer from cough, diarrhea, and delirium. Pleurisy and pneumonia, if present, worsens the prognosis⁹.

Unani scholars could envision and comprehend their sources and reservoirs, modes of transmission of infections, and potential causes of infections turning into epidemics. The theories and observations closely resemble the contemporary knowledge of infections, which reinforce the fact that Unani medicine can play a significant role in

combating current health problems. Among many polyherbal drugs, *Tiryaq e Arba* is a formulation that is used in Unani system of medicine and is being prescribed from centuries with great reputation to boost immunity so that one should have the inner strength to fight epidemics.

Tiryaq e Arba

As its name suggests, *Tiryaq* means antidote and *Arba* means four. The formulation consists of four ingredients and therefore it is called *Tiryaq e Arba*. It is also known by the name of "Tiryaq Sagheer" as per the book "*Bayaz Khas/ ilajul amraz*" of Hakim Shareef Khan, translated by Hakeem Mohammad Kabiruddin¹⁰. The composition of the formulation is as follows¹¹:

Table 1: Formula of Preparation of *Tiryaq e Arba*

S.No.	Name	Botanical Name	Quantity
1.	Juntiyana	<i>Gentiana lutea</i> L.	1 part
2.	Zarawand Taweeel	<i>Aristolochia longa</i> L.	1 part
3.	Mur Makki	<i>Commiphora myrrha</i> (Nees) Engl.	1 part
4.	Habb ul Ghar	<i>Laurus nobilis</i> L.	1 part
5.	Honey or Sugar		Q.S.

Method of Preparation

Tiryaq is a semi solid preparation which comes in *Majoon* category. For any semi solid preparation, *Qiwam* (base) of different consistencies (tar) is generally made. It depends on the nature of ingredient drugs to be used. The *qiwan* is generally made by adding *Aab* (water), *Araq* (distillate) or *Aab e samar* (fruit juices), etc in any of the bases of purified honey, sugar, candy or jaggery etc and boiled over a low heat till it acquires a required consistency. The bases are generally purified by adding *Aab e lemu* (lemon juice), *Satt e lemu* (lemon extract), or *Shibb e yamani* (Alum) before making *qiwan*. Afterwards the ingredients are mixed in *qiwan* to prepare *Tiryaq*. *Qiwam* for *Tiryaq* is of two tar (consistency). All the dry ingredients, after being ground together and sieved through 80-mesh, are made into a *Sufoof* (powder). When the proper *qiwan* (consistency) forms, the medicinal *sufoof* (powder) is gradually added to it during stirring till both mixes. *Tiryaq* is preserved in glass jar^{12,13,14,15}.

Tiryaq-e-Arba has *Dafa e Sumoom* (antidote) and *Dafa e Tashannuj* (anti-spasmodic) properties and is used in the dose of 3-5 gm with lukewarm water^{16,17}.

Physicochemical standards of *Tiryaq e Arba*

Physicochemical standards of *Tiryaq e Arba* were developed by Central Council for Research in Unani Medicine (CCRUM) and published in *physicochemical standards of Unani formulations*, M/o Health and Family Welfare, Government of India. The details of which are as follows¹⁸:

Table 2: Physicochemical Standards of *Tiryaq e Arba*

Appearance	Semi solid
Colour	Dark Brown
Smell	Like rotten walnut
Taste	Bitter
Alcohol soluble matter	48.6 %
Water soluble matter	75.8 %
Successive extractives	
Pet. Ether (60-80°)	1.55 %
Chloroform	0.75 %
Ethyl alcohol	0.85 %
pH of 1% aq soln	4.7
pH of 10% aq soln	4.4
Bulk density at 20° C	1.30
Total ash	1.15 %
Water soluble ash	0.371 %
Acid insoluble ash	0.763 %
Volatile oils	1.00 % v/w
Total phenolics	0.35 %
Reducing sugars	50.02 %
Calcium	28.2 mg/g of ash
Iron	0.069 mg/g of ash
Lead	0.0450 mg/g of ash
Copper	0.069 mg/g of ash
Mercury	Absent
Cadmium	Absent

Thin Layer Chromatography¹⁸

Extracts	Solvent system	Detection	No. of Spots	Rf. Values
Pet. ether	Pet. Ether	Naked eye visible	2	0.43, 0.51
Pet. ether	Pet. Ether : Chloroform (3:1)	Naked eye visible	6	0.29, 0.55, 0.62, 0.68, 0.83, 0.89
Chloroform	Chloroform (100 %)	-do-	1	0.95
Ethanol	Ethyl acetate : Methanol(1: 19)	-do-	1	0.25

Pharmacological Studies on ingredients of *Tiryaq-e-Arba*

Tiryaq-e-Arba is a well-known formulation for its different pharmacological activities as antiviral, analgesic, anti-inflammatory, anti-spasmodic etc. Some of the known pharmacological actions which are mentioned in Unani literature and proven scientifically are mentioned below:

1. *Juntiyana (Gentiana lutea L.)*

Anti-inflammatory and wound healing

Gentiana lutea L. (Gentianaceae) commonly recognized as Yellow gentian is widely used in the traditional system of medicine as an anti-inflammatory and wound healing agent. Investigations were carried out the effectiveness of alcohol and petrol ether extracts of rhizomes of *Gentiana lutea L.* at 500 and 1000 mg/kg doses per oral in the carrageenan-induced rat paw edema, xylol-induced mouse ear oedema and cotton pellet-induced chronic inflammatory models. Both extracts exhibited notable dose-dependent anti-inflammatory activities in all these models. Both extracts exhibited potent wound healing activity at 300 and 500 mg/kg, per oral, in excision, re-sutured incision and dead space wound models¹⁹.

Antioxidant activity

Gentiana lutea L. root is a remedial herb, traditionally used as a bitter tonic in GIT ailments for better digestive system. The active constituents of *G. lutea* were observed to be secoiridoid bitter compounds as well as several other active compounds causing the pharmacological effects. The purpose of current study was to assess the effects of an extract of Yellow Gentian on lipid oxidation throughout storage of an emulsion. *G. lutea* extracts exhibited remarkable antioxidant activity estimated by DPPH scavenging assay and Trolox equivalent antioxidant capacity (TEAC) assays. An amount of 0.5% w/w *G. lutea* lyophilise was capable to inhibit lipid oxidation throughout storage ($p < 0.05$). A mixture of *G. lutea* with 0.1% (w/w) BSA showed a good synergic impact and beneficial antioxidant activity in the emulsion. Quantitative analysis of HPLC confirmed that *G. lutea* contained secoiridoid-glycosides (gentiopicroside and sweroside) and post column analysis revealed radical scavenging activity of *G. lutea* extract towards the ABTS radical. The results from this study highlight the potential of *G. lutea* as a food ingredient in the design of better food commodities²⁰.

Investigators also observed that *Gentiana lutea L.* extracts also exhibits the inhibitory action on the enzyme myeloperoxidase, as well as the antioxidant activity of these extracts and their relationship with the total polyphenol amount. Extracts were prepared using methanol (100%), aqueous and ethanol-water solutions (96, 75, 50 and 25% v/v) as solvents for extraction. Moreover, isovitexin, amarogentin and gentiopicroside, pharmacologically active constituents of *G. lutea* were examined as potential inhibitors of myeloperoxidase. Antioxidant activity of extracts was

concluded using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging test and also using cyclic voltammetry^{21, 22}.

2. *Habb ul Ghar (Laurus nobilis L.)*

Antioxidant activity

The antioxidant activity of *L. nobilis* leaf extract revealed a powerful activity for different extracts obtained with organic solvents^{23, 24}.

In a study conducted, phytochemical investigations on the infusion of *L. nobilis* were performed by high-performance liquid chromatography (HPLC) with a diode array detector (DAD) and direct electrospray ionization-tandem mass spectrometry. Several flavonoid derivatives were detected. Semipreparative HPLC from the infusion of laurel leaves isolated 10 flavonoid O-glycosides, one flavonoid C-glycoside, catechin, and cinnamtannin B₁. Structures of the isolated compounds were computed based on spectral measurements including high-resolution mass spectrometry spectroscopy and one- and two-dimensional nuclear magnetic resonance techniques. The amount of the flavonoids was also determined by HPLC-DAD. The antioxidant activity of the tea and the isolated compounds was also measured using two different *in vitro* methods: the Briggs Rauscher oscillating reaction test, at a pH like that of the gastric juice, and the Trolox equivalent antioxidant capacity assay, at the pH of blood. It was concluded that infusion of *L. nobilis* leaves contain several flavonol glycosides which have antioxidant property²⁵.

Antiviral activity

The antiviral potential of *Laurus nobilis* leaf ethanolic extracts on forager honeybees naturally infected with BQCV (Black queen cell virus) was evaluated. Total viral loads were reduced even at the lowest concentration tested (1mg/ml). Higher extract concentrations (≥ 5 mg/ml) significantly reduced virus replication. Measuring vitellogenin gene expression as an indicator for transcript homeostasis revealed constant RNA levels before and after treatment, suggesting that its expression was not impacted by the *L. nobilis* treatment. In conclusion, plant secondary metabolites can reduce virus loads and virus replication in naturally infected honeybees²⁶.

A study was carried out to evaluate *in vitro* antiviral study, antioxidant, phytochemical screening of *Laurus nobilis* extract and the best molecules of the *L. nobilis* are identified against adenovirus receptors by molecular docking study. Adenovirus has 52 serotypes, each serotype causes various diseases to humans like respiratory disease, gastrointestinal tract disorder, conjunctivitis. 52 serotypes are divided into subgroups and they mostly affect children. The plant compounds were identified by phytochemical screening. Cytotoxicity assay was performed to find percentage of cell viability at various concentration. The antiviral properties of bay leaf were determined by antiviral inhibition assay. *Laurus nobilis* extract was also used in antioxidant activity test. Major compounds of bay leaf plant were used in docking study. The plant has 4.6% of total antioxidant and 1.320 nm

reducing power activity by antioxidant methods. *L. nobilis* extract showed an antiviral activity at 100 μ l/ml of plant extract had 60.5% of the cell protection²⁷.

Immunomodulatory activity

A study was conducted in Algeria on *Lauris nobilis* for essential oil of this plant. The essential oil extraction was performed by steam distillation, the yield obtained from leaf was (1.5 %) by gavage Wistar males rats weight between 100g 80et were infected with *Salmonella* then treated with a dose 1 g / kg of the essential oil. In the day of sacrifice of the rats some parameters were determined: hemoglobin concentration (Hgb); haematocrit (Hct) and lymphocytes (white blood cell). The hematological results obtained reveal the following observations: a significant decrease in the hematocrits: HCT, hemoglobin (HGB), which explains the consequent hemolytic anemia and an increase of the white blood cells especially for the group A, concluding that *Lauris nobilis* showed positive immunomodulatory response²⁸.

3. Zarawand Tawee (Aristolochia longa L.)

Antioxidant activity

A study was conducted to determine polyphenols, flavonoids, and tannins contents of *A. longa* L. extracts. Extracts were prepared from aerial parts (stems and leaves), fruits and tubers by using various solvents with different polarities such as acetone, methanol and distilled water. Acetone extracts from the aerial parts presented the highest contents of polyphenols (525.43 \pm 29.6 μ g/mg) followed by fruit aqueous extract (518.54 \pm 14.93 μ g/mg), while the aerial parts methanol extract shown the highest flavonoid content (52.37 \pm 0.94 μ g/mg) and exhibited the highest antioxidant capacity of DPPH and reducing power (55.04 \pm 1.29 μ g/mL and 0.2 \pm 0.019 mg/mL, respectively), therefore the aerial parts acetone extract showed the highest antioxidant capacity in the β -carotene bleaching inhibition test with 57%²⁹.

A study was conducted to evaluate *in vitro* antioxidant activity and inhibitory potential of organic extracts from *Aristolochia longa* roots against key enzymes linked to hyperglycemia. Antioxidant activity was performed using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals and ferric reducing/antioxidant power (FRAP) methods. The Glucosidase and Galactosidase inhibitory activities were investigated using an *in vitro* model. Moreover, phytochemical analysis of tested extracts was carried out. The aqueous fraction of this herb exhibited the highest antioxidant activity for both DPPH and ABTS methods, IC₅₀=125.40 \pm 2.40 g/mL and IC₅₀=65.23 \pm 2.49 g/mL, respectively. However, the ethyl acetate fraction possessed the strongest inhibitory effect towards Glucosidase (IC₅₀=1.112 \pm 0.026 mg/mL). Furthermore, the result showed high levels of phenolic content. The results showed that this plant could be a significant source of medically important natural compounds³⁰.

Immunomodulatory activity

The study was carried out to evaluate the safety of an aqueous extract of *A. longa* by determining its potential of toxicity after oral administration in mice. To explain the mode of action of *A. longa*, the immunomodulatory activity test was equally carried out. For acute toxicity study, aqueous extract of *A. longa* given to adult 'Swiss albinos' mice in single dose of 2.5 g/kg/day did not produced any visible toxic signs or deaths. While in the sub-chronic toxicity study, the *A. longa* extract at doses of 1.25 and 2.5 g/kg/day for 3 and 6 weeks induced atypical locomotion, anorexia,

asthenia, ataxia, diarrhea and urination for the higher dose used. The histopathological examination showed that *A. longa* extract at 1.25 g/kg was not toxic, while at 2.5 g/kg it caused a significant toxicity on the liver, intestine and kidney. The high number of lymphocytes noted in the different organs indicated that it was an immune activity. In fact, when tested against SRBC, there was a statistically significant increase of "hemagglutinating antibody titer" and insignificant increase, in "delayed type hypersensitivity" response in mice treated by the non-toxic dose of *A. longa* (1.25 g/kg) compared to control group. It was concluded that administration of the aqueous extract of *A. longa* at saturation limit dose (2.5 g/kg) produced severe and irreversible renal toxic effects in mice induced by a high immunostimulant activity³¹.

4. Mur Makki (*Commiphora myrrha* (Nees) Engl.)

Antioxidant activity

A study was conducted for the phytochemical, nutritional, mineral contents and *in vitro* antioxidant activity of *Commiphora myrrha* (Nees) Engl. Preliminary phytochemical results indicated that the plant contain phenolic compounds, flavonoids, tannins, glycosides, alkaloids, terpenoids and quinines. Secondary Metabolites were estimated quantitatively, the highest concentration of tannins 3677.1 \pm 2.15 mg/100g and then for alkaloids 1880 mg/100g, sterols 155.215 \pm 1.00 mg/100g, and Flavonoids 47.266 \pm 0.013 mg/100g and phenolic compounds 30.647 \pm 2.481 mg/100g. Nutritional Profiling, minerals and antioxidant activity were determined. The ability of isolated glycosides and flavonoids to scavenge hydrogen peroxide were determined according to the phosphomolybdenum method and exhibited lower reducing power and scavenging ability than ascorbic acid which conclude that the plant has antioxidant activity³².

The antioxidant and antimicrobial potential of methanol (ME-OH), ethyl acetate (ETOAC) crude extracts and essential oil (EO) of *Commiphora myrrha* resin were investigated. The major constituents of the essential oil identified from the resin of *C. myrrha* were α -elemene (12.86%), 7-isopropyl-1,4-dimethyl-2-azulenol (12.22%), curzerene (11.64%), and germacra-1(10)7, 11-trien-15-oic acid, 8,12-epoxy-6-hydroxy- β -lactone (6.20%). In both DPPH scavenging and Fe²⁺ chelating assays, the ME-OH extract exhibited the highest activity compared to ETOAC extract and EO. Concerning the reducing power ability, EO was superior to Me-OH and ETOAC extracts. The Me-OH extract manifested the highest potential of antimicrobial activity against the tested bacterial and yeast microorganisms, while ETOAC extract and EO showed moderate or no potential antibacterial activity. The Me-OH extract exhibited the highest antioxidant and antimicrobial activity as compared to ETOAC and EO. It is concluded from the present study that besides its traditional use, the *C. myrrha* resin could be used as a natural source for antioxidant and antimicrobial compounds for possible applications in food and nutraceutical industries³³.

Ethanol extract of *C. molmol* exhibit antioxidant activity on *in vitro* study due to the presence of 23 phenolic and flavonoid contents³⁴.

Anti-inflammatory activity

C. myrrha extract exhibit anti-inflammatory effect as evident by decrease in volume of paw edema induced by formalin in rats probably due to an inhibition of release of inflammatory mediator PGs^{35,36}.

Conclusion

It is a likely possibility that epidemics will continue to occur, and with the emergence of new organisms, may be more aggressive than ever. Hence, the need arises to develop new effective methods of infection control that are accessible to the maximum population. *Tiryaq e Arba*, the Unani formulation described in this manuscript is cheap, easy to administer and available in most parts of the world. Through this review it can be easily understood that more proactive researches on Unani medicines can generate credible evidence regarding their role in health promotion and disease prevention and can be helpful for the masses.

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