WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 6.647

Volume 6, Issue 9, 256-264 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL INVESTIGATION OF EXTRACT OF *EMBLICA OFFICINALIS* L. AND *TRIGONELLA FOENUM GRAECUM* L. (ETT01)

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Article Received on
14 July 2017,

Revised on 04 August 2017,
Accepted on 25 August 2017,

DOI: 10.20959/wjpps20179-9835

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ABSTRACT

The study was carried out for determination of chemical constituents of two plants *Emblica officinalis* L. and *Trigonella foenum graecum* L. These two plants are well known and have great chemical value for leading research. In phytochemical analysis of *ETT01 (combine)*, fruits extract showed the presence of alkaloids, reducing sugar, tannins and flavonoids and absence of fixed oil, proteins, resins and saponins. Its seeds extract showed the presence of alkaloids, reducing sugar, flavonoids, proteins, resins, saponin and tannins and absence of fixed oil. Combine phytochemical analysis of *ETT01* extracts showed the presence of alkaloids, carbohydrates, flavonoids, proteins and tannin and absence of fixed oil, resin and saponins. For the first time phytochemical analysis of *ETT01 (combine)* was done. This research finding may be beneficial for future research.

KEYWORDS: Herbal formulation, *Emblica officinalis* L., *Trigonella foenum graecum* L., organoleptic evaluation, phytochemical.

INTRODUCTION

Plants are frequently used in therapeutics from times immemorial. The documentation of therapeutic utility of plants can be seen from vedic period. Now a day's therapeutic utility of many plants are identified. Among of them medicinal plants *Emblica officinalis* L. and *Trigonella foenum graecum* L. are one of the important plants. WHO encourages, recommends and promotes herbal/traditional remedies in natural health care programs because these drugs are easily available at low cost, safe and people have faith in them. The

WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards.[1]

Emblica officinalis L. (Euphorbiaceae) is very popular medicinal plants today. It is found in Pakistan, Uzbekistan, Sri Lanka, India, South East Asia, China and Malaysia. Phytochemical investigations of fruits of plants showed that it has a high concentration of polyphenol with low and high molecular weight gallotannins.[2] It is one of the most extensively studied plant and reports suggest that it contains tannin, alkaloids, ascorbic acid and phenolic compounds.[3,4] Although other parts of this plant are also used for different purposes. It possess antiviral, antibacterial, antioxidant,[5] antiallergy, antidiabetic,[6] anti-inflammatory[7] and antimutgenic properties.[8] The fruits are useful in treating asthma, diarrhea,[9] bronchitis, fever and cardiac disorders. The fruit is the richest source of vitamin C and is used as a diuretic, laxative, hair dye and also cures insomnia. Its powder and oil are used traditionally in Ayurvedic applications for the treatment of scalp. Its powder improves immunity and gives physical strength. It improves complexion and removes wrinkles. It is also used to treat constipation and is used as a cooling agent to reduce the effects of sun strokes and sun burns.[10-11]

Trigonella foenum graecum L. (Fabaceae) commonly known as Fenugreek. It is used both as herb and as a spice. Fenugreek has many medicinal and culinary purposes involving the seeds and the leaves. Fenugreek seeds are a rich source of the polysaccharide galactomannan.[12] They are also a source of saponins such as; diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens. It also contains alkaloids, flavonoids, saponins, amino acids, tannins and some steroidal glycosides and protein. It is beneficial for atherosclerosis, allergy, constipation, diabetes,[13] high cholesterol and hypertriglyceridemia.[14] Its seeds have been used in many traditional medicines as a laxative, digestive, anticancer[15] and as a remedy for cough and bronchitis. Its seeds added to cereals and wheat flour (bread) or made into gruel, given to the nursing mothers may increase breast milk production. The present study involves standardization of herbal formulation (*ETT01).*

MATERIAL AND METHODS

Identification and collection of plant material

The dried fruits of *Embilica officinalis* and *Trigonella foenum graecum* seeds purchased from herbal market of Karachi district (Centre). The material was identified and authenticated by

Prof. Dr. Ghazala H. Rizwani, (Mertiorious), Department of Pharmacognosy, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan.

Plant extract

Embilica officinalis fruits (1.5 kg) were cleaned and crushed into small pieces and seeds were removed. *Trigonella foenum graecum* (1 kg) dried and clean seeds were taken as such. The material was soaked in methanol at room temperature for 10 to 15 days respectively. After this period, the extract was filtered through Whatman filter paper No. 1. The filtrate was evaporated under vacuum using rotary evaporator (Buchi Rotavapor R-200) at 40 ± 2°C.

Organoleptic evaluation

Organoleptic evaluation refers to the evaluation of formulation by color, taste and odor.

Phytochemical analysis

For phytochemical screening, *ETT01* extracts were treated with various different chemical reagents individually and in combine form. The extracts were tested for the presence of bioactive compounds by using standard methods.[16,17]

Detection of alkaloids

Dragendraff's test: 1 or 2 drops of freshly prepared dragendroff's reagent was added to few ml of filtrate and then observed for formation of yellow or orange precipitate.

Detection of carbohydrate

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicates the presence of reducing agent.

Benedict's test: Crude extract was mixed with 2ml benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of carbohydrates.

Mohlish's test: In 2ml of filtrate, two drops of alcoholic solution of alpha naphthol are added, the mixture was shaken well and then 1ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. Formation of violet ring indicates the presence of carbohydrates.

Detection of flavonoids

Ferric chloride test: Extract was boiled with water and filtered to 2ml of the filtrate, two drops of freshly prepared ferric chloride soln. was added. The presence of phenolic hydroxyl group is confirmed by green, blue or violet colorations.

Alkaline reagent test: Extract (2ml) was dissolve in 10% aqueous sodium hydroxide soln. if gives yellow color, a change of color from yellow to colorless on addition of dilute HCL, Indicates the presence of flavonoids.

Lead acetate test: Extract was treated with a few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of fixed oil

Copper sulphate test: Blue color was observed when extract mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Detection of protein

Ninhydrin test: Crude extract when boil with 2ml of 0.2% soln. of ninhydrin, violet color appeared suggesting the presence of amino acid and protein.

Detection of resin

Acetone water test: In 1ml of extract add few drops of acetone water and water and then shake it. After shaken, turbidity indicates the presence of resin.

Detection of saponin

Frothing test: Extract diluted with 20ml distilled water and shaken in graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

Foam test: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for presence of saponins.

Detection of tannin

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride has to be added. Formation of white precipitate indicates the presence of tannins.

Florescence analysis

Powdered of *ETT01* were subjected to analysis under UV light after treatment with various chemicals. Three parameters were taken into account i.e., observation under long wave length UV, short wavelength and normal day light.

RESULT

The organoleptic examination of fruit of *Emblica officinalis* and seed of *Trigonella foenum graecum* was tabulated in Table 1.

In phytochemical analysis of *ETT01* fruits extract showed the presence of alkaloids, reducing sugar, tannins and flavonoids and absence of fixed oil, proteins, resins and saponins and seeds extract showed the presence of alkaloids, reducing sugar, flavonoids, proteins, resins, saponins, tannins and absence of fixed oil. Combine phytochemical analysis of *ETT01* extracts showed the presence of alkaloid, carbohydrates, flavonoids, proteins and tannin and absence of fixed oil, resin and saponins (Table 2).

The florescence analysis of *E. officinalis* fruit and *T. foenum graecum* seed and combine showed different color when treated with different solvents (Table 3-A, 3-B and 3-C).

Table 1: Organoleptic evaluation of *Emblica officinalis* and *Trigonella foenum graecum.*

Organoleptic evaluation	***Emblica officinalis***	***Trigonella foenum graecum***
	Fruit	**Seed**
Color	Black	Yellowish brown
Odor	Unique or characteristic	Pleasant smell
Taste	Bitter	Bitter and mucilaginous
Texture	Rough	Rough
Size	1.4 cm	0.3cm
Shape	Irregular	Parallel plane
Fracture	Weak	Hard
External marking	Irregular surface	Line in the middle

Table 2: Phytochemical screening of *Emblica officinalis* fruits extract (a), *Trigonella foenum graecum* seeds extract (b) and their combine extracts (ab).

Reagents	***A***	***B***	***AB***
Detection of Alkaloids			
Dragendraff's test	+	+	+
Detection of Carbohydrate			
Benedicts	+	-	+
Fehling's test	+	+	+
Molisch test	+	+	+
Detection of Flavonoids			
Alkaline reagent	-	-	-
Ferric chloride	+	+	+
Lead acetate	+	+	+
Detection of Fixed oil			
Copper sulphate test	-	-	-
Detection of Proteins			
Ninhydrin test	-	+	+
Detection of Resin			
Acetone water test	-	+	-
Detection of Saponin			
Foaming test	-	+	-
Frothing test	-	+	-
Detection of Tannin			
Gelatin test	+	+	+

+sign = present

-sign = absent

Table 3(A): Florescence analysis of *ETT01* fruits extract.

Solvents	**Day light**			**Short wave length**			**Long wavelength**		
	0min	**30min**	**48hrs**	**0min**	**30min**	**48hrs**	**0min**	**30min**	**48hrs**
NaOH	*Black*	*Black*	*Yellow*	*Light brown*	*Dark green*	*Yellow*	*Black*	*Black*	*Black*
KOH	*Black*	*Black*	*Orange*	*Light brown*	*Dark green*	*Light green*	*Black*	*Dark brown*	*Black*
HCl	*Black*	*Light green*	*Light brown*	*Light green*	*Light green*	*Green*	*Black*	*Brown*	*Black*
H_2SO_4	*Black*	*Black*	*Black*	*Light green*	*Dark green*	*Black*	*Black*	*Black*	*Black*
Dis. Water	*Black*	*Light brown*	*Light brown*	*Light green*	*Light green*	*Dark green*	*Black*	*Brown*	*Black*
Methanol	*Black*	*Black*	*Black*	*Light green*	*light green*	*Green*	*Black*	*Brown*	*Black*

Table 3(B): Florescence analysis of *ETT01* seeds extract.

Solvents	Day light			Short wave length			Long wavelength		
	0min	30min	48hrs	0min	30min	48hrs	0min	30min	48hrs
NaOH	*Brownish green*	*Light green*	*Light brown*	*Light green*	*Light green*	*Light green*	*Dark brown*	*Brown*	*Dark green*
KOH	*Brown*	*Greenish brown*	*Light brown*	*Light green*	*Light green*	*Light green*	*Brownish green*	*Dark brown*	*Black*
HCl	*Brown*	*Black*	*Dark brown*	*Light green*	*Dark green*	*Green*	*Dark brown*	*Dark brown*	*Black*
H_2SO_4	*Black*	*Black*	*Black*	*Dark green*	*Brown*	*Black*	*Dark brown*	*Dark brown*	*Black*
Dis. Water	*light brown*	*Light brown*	*Light brown*	*Light green*	*Dark green*	*Green*	*Dark brown*	*Dark brown*	*Black*
Methanol	*Off white*	*Yellowish brown*	*Light brown*	*Light green*	*Light green*	*Green*	*Brown*	*Brown*	*Black*

Table 3(C): Combine florescence analysis of *ETT01* extract.

Solvents	Day light			Short wave length			Long wave length		
	0min	30min	48hrs	0min	30min	48hrs	0min	30min	48hrs
NaOH	*Black*	*Black*	*Light brown*	*Greenish brown*	*Dark green*	*Light green*	*Dark brown*	*Black*	*Dark brown*
KOH	*Black*	*Black*	*Light brown*	*Dark green*	*Dark green*	*Light green*	*Dark brown*	*Black*	*Dark brown*
HCl	*Black*	*Black*	*Green*	*Dark green*	*Dark green*	*Green*	*Dark brown*	*Black*	*Black*
H_2SO_4	*Black*	*Black*	*Black*	*Greenish brown*	*Brown*	*Black*	*Black*	*Black*	*Black*
Dis. Water	*Cream*	*Milky*	*Light brown*	*Greenish yellow*	*Light green*	*Brown*	*Dark brown*	*Brown*	*Black*
Methanol	*Milky*	*Light brown*	*Light brown*	*Light green*	*Dark green*	*Green*	*Dark brown*	*Black*	*Black*

DISCUSSION

Organoleptic evaluation is a technique of qualitative evaluation based on the morphological profile of whole drug.[18] The phytochemical analysis revealed that *ETT01* contains tannins, alkaloids, carbohydrates, resins, saponins, proteins and flavonoids. Due to the presence of these phytochemicals it possess antioxidant, antiviral, anticancer, antibacterial and anti-inflammatory activity.[19-20] It is use in cardiovascular diseases[21] and reduces the risk of atherosclerosis,[22] constipation, high cholesterol, hypertriglyceridemia and diabetes. It is also effective against diarrhea.[23] It has tannins which is very much astringent in nature and tannins has high potential treating intestinal disorders such as diarrhea and dysentery.[24]

CONCLUSION

The phytochemical screening shows that *ETT01* extract contains alkaloids, tannins, and flavonoids, carbohydrates, proteins, resins and saponins which are popular phytochemical constituents. For the first time phytochemical analysis of *ETT01* (combine) was done. This research finding may be beneficial for future research.

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