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Pharmacognostical evaluation of *Methika* (*Trigonella foenum graecum* Linn.) seeds

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Abstract
Introduction: *Trigonella foenum graecum* L. known as *Methika* (Family Papillionaceae) is an annual herb being used as culinary and traditional medicine from ancient time.
Methods: The present study was taken to determine the prerequisite pharmacognostic standards like organoleptic or morphological characteristics, microscopic studies, physiochemical evaluations, phyto-chemical screening, HPTLC of the powdered *Methika* seeds for evaluating the seed material.
Observations & Results: The microscopic study showed outermost layer of testa, linea lucida, columella, endosperm layer with aleurone grains and mucilage respectively. Phytochemical analysis showed various of phyto-constituents like alkaloid, steroids, tannins, saponins, amino acids, coumarins and amino acid. TLC of aqueous extracts of Methika showed 10 spots under long UV rays.
Discussion: The determination of these characteristics will aid future investigators in their pharmacological evaluation for correct identification and standardization of *Methika* seeds.

Keywords: Pharmacognostic, *Trigonella foenum graceum*, Seeds, Phytochemical, HPTLC, Evalution

Introduction
Plants are always an exemplary source of drugs from the development of the mankind. 80-90% of the World population depends upon the traditional herbal medicine in developing and developed countries[1] and it is estimated that about 25% of all modern medicine is derived directly or indirectly from higher plants [2]. India is the country of vast biodiversity and traditional knowledge for using herbal medicines to cure many ailments in various cultures and tribes. Standardization and quality control of the raw materials and the final product is an important aspect for quality, safety and efficacy of the plant drugs [3]
Trigonella foenum graecum Linn. is an annual herb, (Papilionaceae) commonly known as *Methika* being used as culinary and traditional medicine from Ancient time. *Trigonella foenum graecum* Linn. is native to Eastern Europe and parts of Asia but now widely cultivated almost all over the world for its leaves and seeds, which are commonly used as leafy vegetables and condiments, respectively [4].
Methika seeds are bitter, mucilaginous, diuretic, astringent, carminative, tonic galactogogue and aphrodisiac & are useful in fever, vomiting, flatulence, colic, dysentery, diarrhea, dyspepsia, diabetes, lumbago, puerperal disorders, anorexia, cough and bronchitis [5]. *Methika* is possessed with insecticidal, antibiotics, hypoglycemic, fungi toxic, antineoplastics, antinociceptives, mild smooth muscle relaxant, antibacterial, wound healing hypocholesterolemic, cardiovascular, antifertility, anti-androgenic, hypolipidemic, & antitumour properties [6].

Materials and Methods
Collection of the Sample
The test drug, *Methika* seeds (*Trigonella foenum graecum* Linn.) was purchased from the local market of Hassan. The authentication was done at Department of Dravyaguna, SDM College of Ayurveda & Hospital, Hassan (Karnataka) and a voucher specimen maintained in the same laboratory. The collected seeds were then grinded to powder and stored by keeping in air tight glass vessels for further use.

Macroscopy
Macroscopic features of the seeds of *Trigonella foenum graecum* Linn. were observed under Stereo microscope (Zeiss Stemi) and the characters recorded with reference to seed characters reported in literature.

Microscopy
Transverse section of the seed was taken and observed for their characteristic features [7].

Powder characteristics
Minimum quantity of fine seed powder was mounted on a microscopic slide, stained with safranin, characters were observed under trinocular microscope (Zeiss AXIO) [8].

Analytical Characteristics
Physicochemical constants such as total ash, acid insoluble ash and water soluble ash; water and alcohol soluble extractive values were calculated according to the methods described in Pharmacopoeia of India [9]. Preliminary phytochemical analysis of aqueous extracts of *Methika* seeds was performed [10]. The test were performed to investigate presence of Alkaloid, Carbohydrates, Steroids, Tannins, Saponins, Proteins, Amino acids, Starch, Triterpenoids, Coumarins, Carboxylic acid, Resin, Quinone, Phenols and Flavonoids.

High Performance Thin Layer Chromatography
Extract preparation
1g of *Methika churna* powder was extracted with 10 ml of *alcohol*. 3, 6 and 9µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in n-hexane: Ethyl acetate (8.0: 2.0). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm. R_f, colour of the spots and densitometric scan were recorded [11, 12].

Observation and Results
Organoleptic Characters
Methika seeds powder is yellowish in colour, bitter in taste and with the characteristic odour.

Macroscopic study of the seeds

Fig 1: Seeds enlarged

Fig 2: Macroscopy of seeds

The seeds are oblong, flattened or irregularly rhomboidal with deep furrow running obliquely form one side dividing the seed into a large and smaller part; 3-5 mm long, 1.5-3 mm wide, and about 2mm wide. Seed powder is brownish white in colour; bitter in taste and with the characteristic odour.

Microscopic study of the seeds
The transverse section of *Methika* shows the outer seed coat, which is irregular in shape. The seed consists of three parts; testa, endosperm and cotyledon occupying the major portions of the seeds. The detailed T.S of the seeds shows the outermost layer of the testa, covered by cuticle, composed of a single row of highly thick walled, cylindrical palisade like lignified cells with the conical projections, through which a light line, linea lucida extends across along with the column cells. The endospermic layer consists of rectangular to polygonal layer thick walled cells containing aleurone grains, along with several layers of thin walled mucilaginous cell of various shape and size. Cells of cotyledons contain aleurone grains and oil globules.

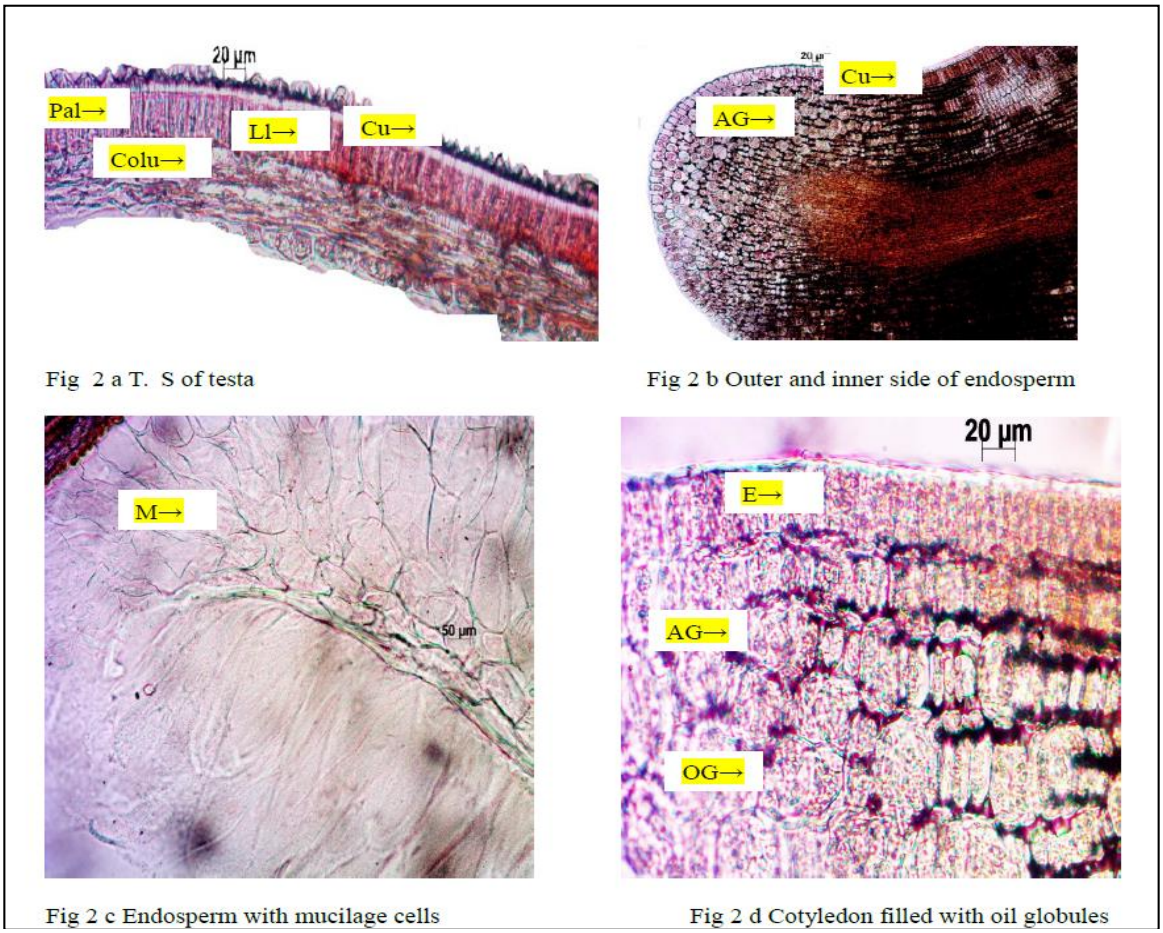


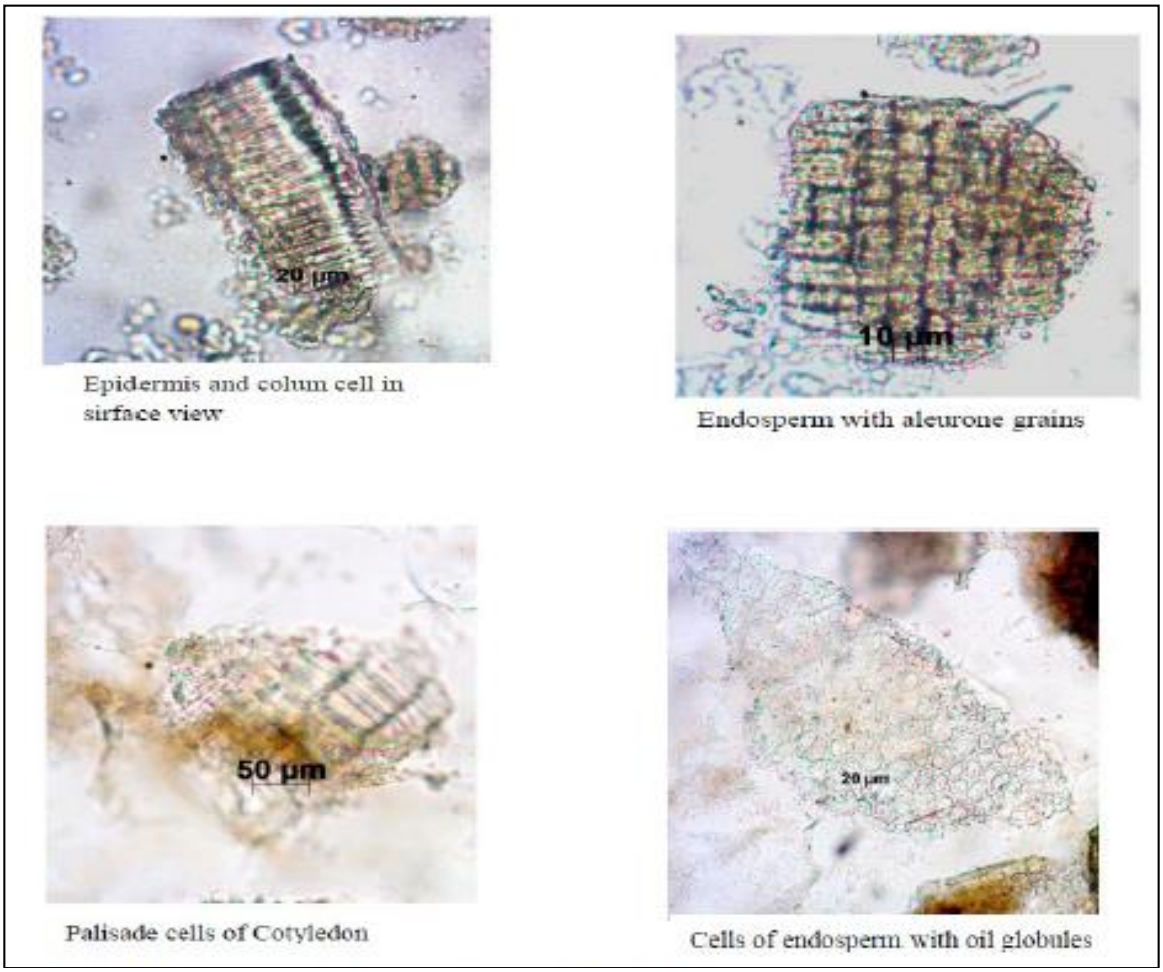
Fig 2 a T. S of testa

Fig 2 b Outer and inner side of endosperm

Fig 2 c Endosperm with mucilage cells

Fig 2 d Cotyledon filled with oil globules

Pal- Palisade layer, colu- columella, Ll-Linea lucida, Cu- Cuticle, Ag-Aleurone grains, M- Mucilage, OG- Oil Globules, Ag- Aleurone Grains



Epidermis and colum cell in sirface view

Endosperm with aleurone grains

Palisade cells of Cotyledon

Cells of endosperm with oil globules

Fig 3: Powder microscopy of *Trigonella foenum graecum*

The fine powder of *Methika* is yellow in colour; shows epidermis and column cells of testa, endospermic cells containing the aleurone grains, mucilage layers; fragments of palisade cells of cotyledons, aleurone grains & oil globules.

Physiochemical Analysis

Table 1: Results of standardization parameters of *Meethika churna powder*

Parameters	Observed Values(n=3)
Foreign organic matter	1.63±0.03
Loss on Drying	7.57±0.03
Ash	5.67±0.23
Acid insoluble ash:	0.00±0.00
Water Soluble Ash	4.73±0.05
Ethanol soluble extractive:	10.04±0.01
Water soluble extractive:	30.49±0.01

Table 2: Results of preliminary phytochemical screening of aqueous extract of *Meethika churna*

S.N	Phytoconstituents	Inference(+ present, - absent)
1	Alkaloid	+
2	Steroid	+
3	Carbohydrate	+
4	Tannin	+
5	Flavanoids	-
6	Saponins	+
7	Terpenoid	-
8	Coumarins	+
9	Phenols	-
10	Carboxylic acid	-
11	Amino acids	+
12	Resin	+
13	Quinone	-

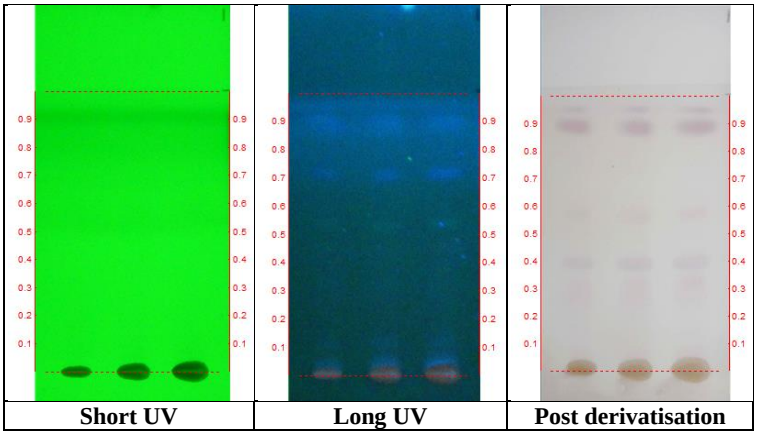


Fig 4: HPTLC Photodocumentation of sample of *Methika churna* Track 1: *Methika churna* - 3µl Track 2: *Methika churna* - 6µl Track 3: *Methika churna* - 9µl Solvent system: n-hexane: Ethyl aetate (8:2)

Table 3: Rf values of sample of *Methika churna*

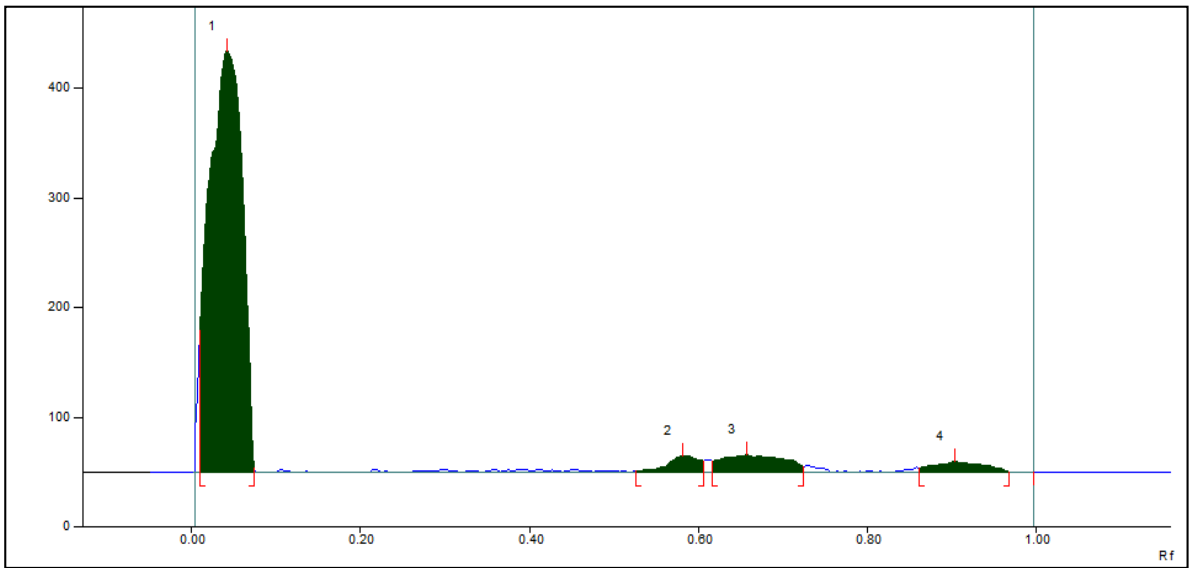
Short UV	Long UV	Post derivatisation
-	0.13 (F. blue)	-
-	-	0.30 (Purple)
-	-	0.40 (Purple)
-	0.54 (F. blue)	-
-	-	0.57 (Purple)
-	0.72 (F. blue)	-
-	0.90 (F. blue)	0.90 (Purple)

*F - fluorescent

Table 4: Showing the peaks detected during densitometric scan of *Trigonella foenum graecum* seeds at 254 nm Track 3, ID: Meethika Exuact

Track 3, ID: Meethika extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	129.1 AU	0.04 Rf	383.4 AU	90.20 %	0.07 Rf	3.9 AU	10862.0 AU	86.54 %
2	0.53 Rf	0.9 AU	0.58 Rf	15.5 AU	3.64 %	0.61 Rf	10.5 AU	404.1 AU	3.22 %
3	0.62 Rf	10.5 AU	0.66 Rf	15.7 AU	3.68 %	0.73 Rf	5.5 AU	862.6 AU	6.87 %
4	0.86 Rf	4.2 AU	0.90 Rf	10.5 AU	2.48 %	0.97 Rf	0.4 AU	422.3 AU	3.36 %

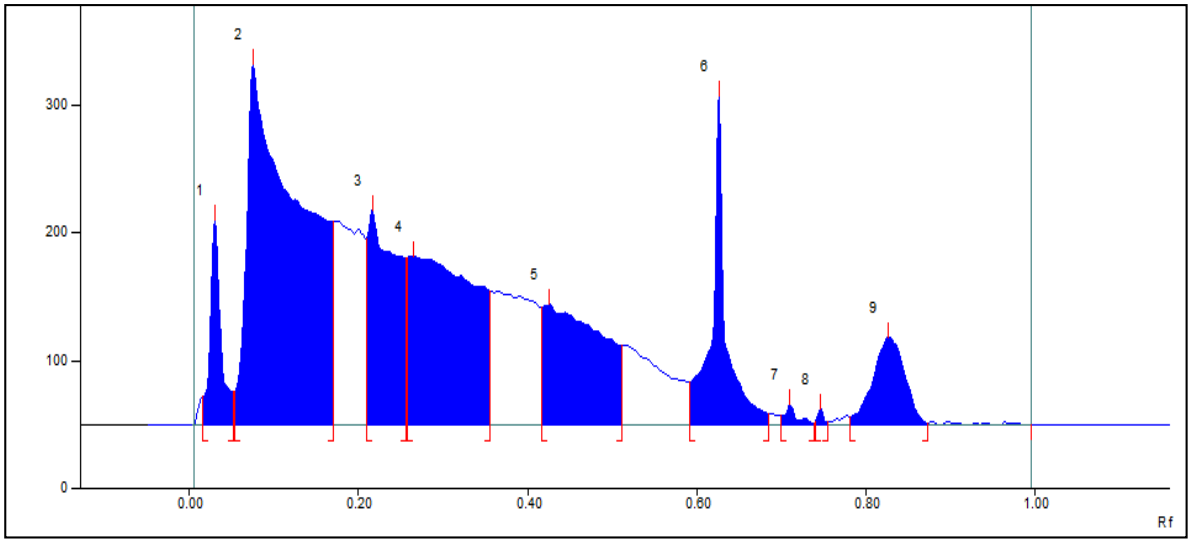


Trigonella foenum graecum seeds at 254 nm

Table 5: Showing the peaks detected during densitometric scan of Methika seeds at 366 nm

Track 3, ID: Meethika extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	22.0 AU	0.03 Rf	159.7 AU	13.46 %	0.05 Rf	25.6 AU	1480.0 AU	4.13 %
2	0.05 Rf	25.7 AU	0.08 Rf	281.1 AU	23.69 %	0.17 Rf	58.4 AU	12877.0 AU	35.92 %
3	0.21 Rf	145.1 AU	0.22 Rf	167.3 AU	14.10 %	0.26 Rf	30.2 AU	4201.6 AU	11.72 %
4	0.26 Rf	130.5 AU	0.27 Rf	131.2 AU	11.05 %	0.36 Rf	04.4 AU	7415.6 AU	20.68 %
5	0.42 Rf	91.9 AU	0.43 Rf	93.8 AU	7.91 %	0.51 Rf	61.5 AU	4719.2 AU	13.16 %
6	0.59 Rf	33.7 AU	0.63 Rf	256.9 AU	21.65 %	0.69 Rf	9.1 AU	3077.6 AU	8.58 %
7	0.70 Rf	7.6 AU	0.71 Rf	15.8 AU	1.33 %	0.74 Rf	1.1 AU	162.0 AU	0.45 %
8	0.74 Rf	2.0 AU	0.75 Rf	12.3 AU	1.04 %	0.76 Rf	2.3 AU	63.8 AU	0.18 %
9	0.78 Rf	6.5 AU	0.83 Rf	68.5 AU	5.77 %	0.87 Rf	1.6 AU	1855.7 AU	5.18 %



Trigonella foenum graecum seeds at 366 nm

Discussion

Evaluation of plants by Pharmacognostical study serves important role for standardization. The study of pharmacognostical parameters provides a direction to identify the plants, detect adulterants and provides diagnostic features. Macroscopic and Microscopic evaluation are important for establishing the proper identity, and genuity of the drugs. Organoleptic, Macroscopic & Microscopic features recorded was found as per Ayurvedic Pharmacopeia of India.

Analytical study shows some of the parameters like Loss on drying, total ash value etc. which can be used as standard for further study regarding the purity and genuity of the drugs. Foreign matter represents the other material than the used part of the drug itself. The foreign matter of the *Mehtika* seeds was

found to be 1.63±0.03%. The loss on drying of any drug is related to the moisture content. An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects which affects the quality of the drugs and hampers its preservation as well. The loss on drying of the drugs *Methika* seeds was determined at 105 degree Celsius & was found to be 7.57±0.03%.

The residue remaining after incineration of plant materials is Ash content of the drug, which includes inorganic salts such as carbonates, sulphates, phosphates, silicates etc. This includes both the physiological ash derived from the plant tissue itself & non physiological ash which is the residue of the extraneous matter (soil & sand), adhering to it or deliberately added to it in the form of adulteration. Total ash is the measure of amount of plant material after ignition of the drugs. Acid insoluble ash or water soluble ash content is the residue obtained after boiling the total ash either with dilute hydrochloric acid or water which measures the amount of silica and sand matter present in the drug. The results of ash value for Methika seeds showed total ash content of 5.67±0.23%, Acid insoluble ash 0.00±0.00% and water soluble ash to be 4.73±0.05%. The results of water soluble and alcohol soluble extractive values for Methika seeds showed 10.04±0.01% & 30.49±0.01% respectively.

Preliminary Phytochemical tests are used to detect the presence of various organic functional groups, which is indicative of type of phyto-chemicals present in the plants. The phyotchemical analysis of hydro aqueous extracts shows presence of many medically important secondary metabolites type of constituents like alkaloids, steroids, carbohydrates, tannins, saponins, coumarins, amino acid and resins which indicates that *Methika* seeds possess high profile values and can be used to treat various kinds of disease.

Chromatographic study is an important tool in qualitative and quantitative analysis of the drugs. In this study, Thin Layer Chromatography (TLC) and High Performance Chromatography (HPTLC) methods were conducted. The plates developed were visualized in UV-254 nm, 366 nm, and then post derivatised.

From the results obtained no retention factor (Rf) values for *Trigonella foenum graecum* were observed at short wavelength (254nm). At long wavelength, retention factor for *Trigonella foenum graecum* seeds were 0.13(F. blue), 0.54(F. blue), 0.72 (F. blue) & 0.90(F. blue) were observed. In post derivatization retention factor for *Trigonella foenum graecum* seeds were 0.13 (Purple), 0.40 (Purple), 0.57 (Purple) and 0.90 (Purple).

Densitometric scan at 254nm, *Trigonella foenum graecum* seeds showed 4 peaks at Rf value 0.01, 0.53, 0.62 & 0.86 having highest area in Rf 0.01 which is 86.54%. Similarly at 366 nm reveals 9 peaks at Rf value 0.02, 0.05, 0.21, 0.26, 0.42, 0.59, 0.70, 0.74, 0.78 having high area in 0.05 Rf(35.92%) and 0.26 Rf(20.68%).

Conclusion

The pharmacogonostic and analytical study have confirmed the authenticity and purity of the drugs, the values reported could be used as supplement tool for the Standardization of the Methika seeds. The determination of these characteristics will aid future investigators in their pharmacological evaluation for correct identification and standardization of Methika seeds.

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