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PHYTOCHEMICAL ANALYSIS OF SOME HEPATOPROTECTIVE HERBS AGAINST HEPATIC DISEASES

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ABSTRACT Medicinal plants are most accepted in various ancient system of medicines because of pharmacological properties and their potential effects in the biological system. Phytochemicals are have more protective and disease preventive qualities. The main objective study was to screen the hepatoprotective activities of *Trachyspermum ammi (L.)*, *Trigonella foenum- graecum* L., and *Piper nigrum L.* The plant extracts analysed for their phytochemical content with standard procedures. Qualitative phytochemical assays were used to detect the presence of alkaloids, carbohydrates, flavonoids, resins, terpenoids, organic acids, inorganic acids, phenolic compounds, proteins, oils and fats. Presence of these phytochemicals in these three selected medicinal plant seed extracts indicates the presence of hepatoprotective properties against various hepatic disorders. This study aimed to prove the pharmacological properties. This study experimentally confirm the use of these medicinal plants are consists of hepatoprotective phytochemicals.

KEYWORDS : Hepatoprotective activity, *Trachyspermum ammi (L.), Trigonella foenum- graecum L.,* and *Piper nigrum L.,* qualitative phytochemical standard methods.

INTRODUCTION

Indian medicinal plants used as traditional medicines for various types of diseases. Medicinal plants are the sources of ayurvedic and siddha medicines. Naturally available plant products to prove the beneficial and helpful to improve the health of human body. Usually all parts of the medicinal plants used to prepare the ayurvedic medicines. The recent focus on plants research has increased throughout the world and larger in number of evidences were collected to show tremendous potential plants used in various traditional systems. In this modern trend, the researchers have direct at identifying and confirm plant derived substances for the treatment of various diseases. The various parts of the plants such as leafs, fruits, seeds are provide health, nourishment and promoting compounds in human diet. Medicinal plant provide good source for preparation of novel drugs as well as resists diseases, from the emerge of civilization. Traditional medicine is the aggregate knowledge, skills and practical based theories and experiences indigenous to different lifestyle that are used to maintained healthy as well as prevent, diagnose, develop or treat physical and stress disorders. Herbal treatments are the most popular traditional medicine. Herbal medicines includes herbs, herbal materials, herbal preparation and finished herbal products that certain parts of the plants or other plant materials as active ingredients.

The World Health Organization (WHO) has attribute importance on promoting the use of traditional medicine for health care [1]. Hence a focus turns on traditional and herbal medicines, especially developing countries, with individual as well as association efforts by national research organisations [2].

The performs the normal metabolic functions of human body as well as biotransformation, detoxification and removal of waste products of many exogenous and endogenous compounds including chemical drugs environmental chemicals. Drug induced hepatotoxicity is the major cause of hepatic diseases, accounting for one 600 to one in 3500 of all hospital admissions [3]. Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several herbal compounds as evidenced from the capacious work on their hepatoprotective potentials [4]. More than seven hundred mono polyhedral compositions from over a hundred different plants are available for use [5].

2.MATERIALS AND METHODS

Trachyspermum ammi (L.) (*Apiaceae*), *Trigonella foenum-graecum L.* (*Fabaceae*) and *Piper nigrum L.* (*Piperaceae*) are annual herbs which are distributed throughout India. These plants are incorporated as one of the ingredients and potential compounds in various preparations available to treat liver ailments. These three plants (*Trachyspermum ammi (L.), Trigonella foenum-graecum L.* and *Piper nigrum. L*) have well- characterized bioactive compounds identified from the aqueous extracts.

2.1. PHARMACOGNOSTIC STUDY

2.1.1. Plant 1: *Trachyspermum ammi (L.)* (Ajwain)

The seeds were examined and evaluated based on colour; odour, taste, and texture etc. Organoleptic characters of the samples were carried out based on the method as described by Siddiqui (1995) [6]. *Trachyspermum ammi (L.)* seeds are small; oval-shaped, seeds are pale brown bitter and pungent taste [7].

Fig - 1. *Trachyspermum ammi (L.) seed*

Fig - 2. *Trigonella foenum-graecum L.* Seeds

2.1.2. Plant 2: *Trigonella foenum-graecum L.* (fenugreek):

The morphological studies were carried out according to the method of (Jyoti Verma, 2013)[8]. Different parameters such as shape, size, colour, odour, taste and identification of the *Trigonella foenum-graecum L.* (fenugreek) seed was studied.

The seeds are oblong, rhomboidal with deep furrow running obliquely from one side, dividing the seed into a larger and smaller part, 0.2-0.5 cm long, 0.15-0.35cm broad, smooth, very hard; dull yellow; seed becomes mucilaginous when soaked in water; odour, pleasant taste and bitter.

2.1.3. Plant 3: *Piper nigrum L.* (Pepper)

The fruit of *Piper nigrum L.* was subjected to macroscopic studies which comprised of organoleptic characteristics viz, colour, odour, appearance, taste, shape, texture etc. These parameters which are considered to be quite useful in quality control were evaluated as per standard methods prescribed by WHO guidelines [9].

Fig – 3. *Piper nigrum L.* Seeds

2.2. NUTRITIVE CONSTITUENT ANALYSIS

The important constituents such as carbohydrates, proteins, fats, vitamins, minerals and water were analyzed (Indrayan, 2005) [10]. Primary phytochemical analysis was carried out according by the methodology of Sofowara 1993.

2.3. Mineral elements analysis:

Mineral elements analysis was carried out by method of Evans & Trease 1989[11] and Harborne 1973 [12]. It is evident that mineral nutrient is important to maintain good health and therefore determination of As, Ca, Fe, Mg, K, Z, Ni, Co, etc., has been added to ayurvedic pharmacopeia of India (The Ayurvedic pharmacopeia 1999) [13].

2.4. Physiochemical analysis:

According to the WHO 2003 [14] approximately 75- 80% of the world's population use plant-based medicines. All plants may not be as useful as claimed or may have more therapeutic properties that are known traditionally and hence proper scientific knowledge is required to investigate an explore the exact standardization of such medicinally important plants [15]. The physiochemical analysis was carried as per the standard method of Pharmacopoeia (1996) [16]. It includes a number of parameters such as physical state, colour, taste, percentage loss on drying of seeds etc.

2.5. PLANT COLLECTION AND EXTRACTION METHODS

The seed samples were locally procured in Vellore. They were collected, identified, and authenticated by Plant Anatomy Research Center (PARC), Chennai and a voucher specimen was deposited at the Herbarium of PARC for future reference [PARC/2017/3560, PARC/2017/3561, and PARC/2017/3562].

Extractions were performed using Soxhlet extractor. About 50 gm of the shade-dried and powdered sample of each seed samples of *Trachyspermum ammi (L.), Trigonella foenum-graecum L.* and *Piper nigrum L.* was taken and extracted in 500 ml of aqueous solution (Double distilled water) by using soxhlet apparatus maintained at 70^{0}C [17].

The concentrated liquid extracts obtained were then transferred to a beaker and kept in hot air oven at 85^{0}C for further drying until the extract was obtained in paste form. 15 gm of the final dark brown and black colour crude extract obtained was stored in an airtight container and kept in a refrigerator at 4^{0}C for further studies.

2.5.1. Phytochemical screening

The phytochemical assessments have been carried out for the above mentioned plant crude extract using the standard methods to identify the components mentioned below.

2.6. QUALITATIVE PHYTOCHEMICALANALYSIS

The extracts were subjected to analysis to identify the presence or absence of various active compounds like phenolic compounds, carbohydrates, flavonoids, glycosides, cardiac glycosides, saponins, alkaloids, steroids, organic acids, quinines, anthroquinones, terpenoids, coumarins, steroids, phlobatannins, resins, tannins, amino acids, oil and fats which was carried out by standard procedure [18,19,20,21,22].

2.6.1. Test for alkaloids

a) Wagner's test

To 0.5 ml of plant extracts the Wagner's reagent was added. (Solution of Iodine in potassium Iodine). A reddish brown precipitate confirms that test as positive.

2.6.2. Test for Phenolic compounds

a) Lead acetate test

To 0.5 ml of plant extracts few drops of 10% lead acetate solution was added. White precipitate indicated the presence of phenolic compounds.

2.6.3. Test for Carbohydrates

a) Benedict's test

0.5 mg of plant extracts was shaken with 2.5 ml of water, filtered and the filtrate was concentrated. To this 1.25 ml of Benedict's solution was added and boiled for 5minutes. Brick red precipitate indicated the presence of carbohydrates.

b) Fehling's test

0.5 ml of plant extracts was hydrolyzed boiling with 5ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. Then few drops of Fehling's solution was added and heated on a water bath for 2 minutes. Reddish- brown precipitate indicated the presence of combined reducing sugars.

2.6.4. Test for Flavonoids

a) Shinoda's test

To 0.5 ml of plant extracts a piece of metallic magnesium was added, followed by addition of 2 drops of concentrated hydrochloric acid. Presence of deep red colouration indicated the presence of flavonoids in the extract.

2.6.5. Test for glycosides

a) Keller killaini's test (Cardiac glycosides)

0.4 ml of glacial acetic acid containing traces of ferric chloride and 0.5 ml of concentrated sulphuric acid were added to the plant extracts carefully. A reddish- brown colour formed at the junction of the two layers and the upper layer turned bluish green indicating the presence of glycosides.

2.6.6. Test for resins

0.5 ml of plant extracts were treated with a few drops of acetic anhydride solution followed by one ml of concentrated sulphuric acid. Resins give colouration ranging from orange to yellow.

2.6.7. Tests for Steroids

a) Salkowski's test (Triterpenoids)

0.5 ml of each extract was treated in chloroform with few drops concentrated sulphuric acid, shaken well and allowed to settle for some time. Red colour at the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicates the presence of triterpenoids.

2.6.8. Test for tannins

a) Lead acetate test

To 0.5 ml of plant extracts, a few drops of 10% lead acetate were added. Precipitate was formed, indicating the presence of tannins.

2.6.9. Test for saponins

1 gm of each portion was boiled with 5ml of distilled water, filtered. To the filtrate, about 3ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins [23].

2.6.10. Test for starch

To 0.5 ml of plant extracts, iodine as reagent was added. Appearance of dark blue colour which disappeared on heating and reappears on cooling indicated presence of starch.

2.6.11. Test for inorganic acids

a) Sulphate test

To 0.5 ml of plant extracts, the lead acetate reagent was added. White precipitates which is soluble in NaOH Indicated the presence of sulphate.

b) Carbonate test

To 0.5 ml of plant extracts, dilute HCl solution was added. Liberation of CO_2 gas indicated the presence of carbonates.

2.6.12. Test for organic acids

a) Malic acid test

To 0.5 ml of plant extracts few drops of 40% $Fecl_3$ solution was added. Formation of yellowish colour indicated the presence of Malic acid.

b) Oxalic acid test

To 0.5 ml of plant extracts, few drops of 1% $KMnO_4$ and dilute H_2SO_4

were added. Disappearance of colour indicated the presence of oxalic acid.

2.6.13. Test for ascorbic acid

To 0.5 ml of plant extracts, 2ml of water, 0.1 gm of sodium bi carbonate and about 20 mg ferrous sulphate were added, shaken and allowed to settle down. A deep violet colour was produced. To this 5 ml of 1 ml of sulphuric acid was added, the colour disappeared showing the presence of ascorbic acid.

2.6.14. Test for phenolic compounds

a) Lead acetate test

To 0.5 ml of plant extracts few drops of 10% lead acetate solution was added. White precipitate indicated the presence of phenolic compounds.

2.6.15. Test for Amino acids

a) Ninhydrin test

To 0.5 ml of plant extracts few drops of 5% Ninhydrin was added and then boiled. Appearance of violet colour indicated the presence of amino acids.

2.6.16. Tests for protein

a) Biuret Test

To 0.5 ml of plant extracts, 4% NaOH solution and few drops of 1% $CuSO_4$ solution were added violet colour appeared, indicating the presence of protein.

2.6.17. Test for oils and fats

A small quantity of the dried plant was pressed between the two filter papers. Oil stain on the filter papers indicated the presence of oils and fats.

2.6.18. Test for coumarins

To 0.5 ml of plant extracts, 10% NaOH solution was added. The appearance of yellow colour indicated the presence of coumarins.

2.6.19. Test for phlobatannins

To 0.5 ml of plant extracts, a solution of 10% ammonia was added. Appearance of pink colour indicated the presence of phlobatannins.

2.6.20. Test for Anthraquinones

To 0.5 ml of plant extracts few drops of 2% HCI was added. Red colour precipitate indicated the presence of Anthraquinones.

RESULTS AND DISCUSSIONS

Fig– 4. Extractions of seed- Soxhlet method

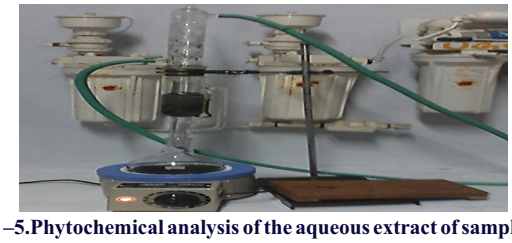


Fig –5.Phytochemical analysis of the aqueous extract of sample -01 (*Trachyspermum ammi (L.)*)

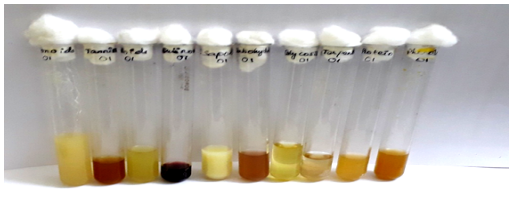


Fig –6.Phytochemical analysis of the aqueous extract of sample -02 *(Trigonella foenum graecum L.)*

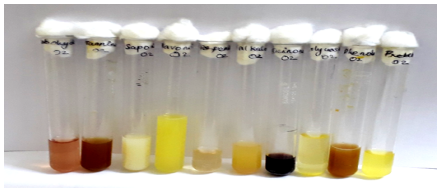
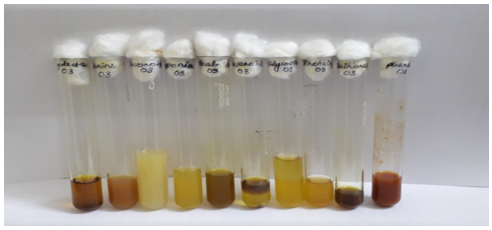
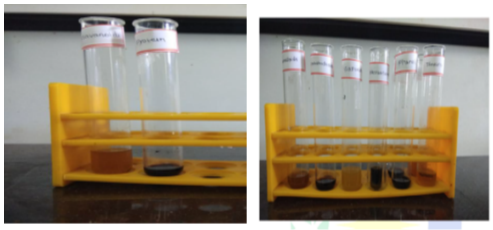


Fig – 7. Phytochemical analysis of the aqueous extract of sample -03 (Piper nigrum L.)



Fig–8. Presence of Alkaloids, Phenols, Flavonoids, Carbohydrates, Protein, Saponins, Steroids, fats & oils



Phytochemical analysis of *T.ammi, T.foenum-graecum* and *P. nigrum*

(Aqueous extracts) - PHYTOCHEMICAL ANALYSIS

The phytochemical analysis was carried out by the standard procedure and the results were tabulated. Phytochemical analysis by the qualitative method revealed the presence of the following compounds.

Table - 1. Phytochemical analysis of *T.ammi, T.foenum-graecum* and *P. nigrum*(Aqueous extracts)

S.NO	PHYTO CHEMICAL ANALYSIS	PLANT SEED EXTRACTS-AQUEOUS		
		TA	TFG	PN
1.	TEST FOR ALKALOIDS			
a.	Wagner's test	++	++	++
2.	TEST FOR CARBOHYDRATES			
a.	Benedict's test	++	++	+
b.	Fehling's test	++	++	+
3.	TEST FOR FLAVONOIDS			
a.	Shinoda's test	++	+	-
4.	TEST FOR GLYCOSIDES			
b.	Keller killaini's test (cardiac glycosides)	-	+	+
5.	TEST FOR RESINS	++	+	-
6.	TEST FOR TERPENOIDS			
a.	Salkowski test	++	++	+
7.	TEST FOR TANNINS			
a.	Lead acetate test	+	+	+
8.	TEST FOR SAPPONINS	+	+	+
9.	TEST FOR STARCH	+	+	+
10.	TEST FOR INORGANIC ACIDS			
a.	Sulphate test	++	+	-
b.	Carbonate test	++	+	-
11.	TEST FOR ORGANIC ACID			
a.	Malic acid test	+	+	+
b.	Oxalic acid test	+	+	+
12.	TEST FOR ASCORBIC ACID	+	-	+
13.	TEST FOR PHENOLIC COMPOUNDS			
a.	Lead acetate test	++	++	++
14.	TEST FOR AMINO ACIDS			
a.	Ninhydrin test	+	+	+
15.	TEST FOR PROTEINS			
a.	Biuret test	+	++	+
16.	TEST FOR OILS AND FATS	+	+	+
17.	TEST FOR COUMARINS	+	-	-

18.	TEST FOR PHLOBATANNINS	-	-	-
19.	TEST FOR ANTHRA QUINONES	-	+	+

++ = strongly present + = moderately present - = absent

Preliminary phytochemical screening was done for three plant seed extracts and the results were incorporated in the **Table. 1** and **Figures: 1 to 8.**

The phytochemical analysis of the aqueous extracts of chosen medicinal plant seeds of *Trachyspermum ammi (L.)*, *Trigonella foenum- graecum (L.)* and *Piper nigrum L.* showed the presence or absence of carbohydrates, alkaloids, flavonoids, triterpenoids, steroids, tannins, phenolic compounds, coumarins, resins, saponins, oils and fats, inorganic acids and ascorbic acids. Flavonoids and tannins are phenolic compounds which act as primary antioxidants or free radical scavengers, anti- inflammatory and anti- carcinogenic. The presence of phenolic compounds may play a role in the prevention of several chronic diseases as reported by Canini, 2007 [24]. The active principles identified are alkaloids strongly present in the aqueous extracts of *T. ammi, T. foenum-graecum* and *P.nigrum*. Carbohydrates are the common major nutrient of all edible seeds. The presence of carbohydrates was confirmed in the samples of *T. ammi* and *T. foenum-graecum* although only moderately present in *P. nigrum*. Flavonoids inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide syntheses, which are involved in free radical generation, thereby resulting in the decrease of the oxidative damage of macromolecules [25]. Flavonoids are strongly present in the *Trachyspermum ammi (L.)*, moderately in the *Trigonella foenum-greacum L.* and absent in *Piper nigrum L.*

The **Table. 1.** represents the qualitative analysis. Glycosides are absent in *T.ammi* and moderately present in *T. foenum-graecum* and *P.nigrum.* Resins strongly present in *T. ammi*, moderately present in *T. foenum-graecum* and absence in *P.nigrum*. The qualitative analysis shows that terpenoids a steroid compound is strongly present in both *T. ammi* and *T. foenum -graecum*, moderately present in *P. nigrum*.

The presence of steroid is in moderate levels in the *T.ammi, T. foenum-graecum* and *P.nigrum* respectively. Tannins, saponins and starch are in moderate or negligible levels in the three samples. *T.ammi* strongly confirmed the presence of inorganic acid and moderate level of organic acid. *T.foenum-graecum* consists of moderate level of inorganic acids and shows the moderate levels of organic acids. *P.nigrum* shows the absence of inorganic acids and presence of moderate level of organic acids.

Ascorbic acid moderately presents in the sample *T. ammi* and *P. nigrum* and absent in *T. foenum- graecum*. Phenolic compounds strongly present in all the three samples. Amino acids are in moderate levels in all the samples, proteins are strongly present in *T. foenum-graecum* and moderately present in the other two samples. Oils and fats are moderately present in all the three samples. The presence of moderate level of coumarins in *T. ammi* are absent in *T.foenum-graecum* and *P. nigrum*. Essential oils of *T. ammi* exhibited good antibacterial, antifungal activities.

The essential oil of the seeds of *T.ammi* has gastro protective, hepatoprotective and analgesic potential, was previously reported by Chauhan, 2012 [26]. Phlobatannins were absent in all the samples and anthra quinones were present in *T.foenum-graecum*, *P. nigrum* and absent in *T. ammi*.

CONCLUSION

Hepatotoxicity and chronic liver damage due to different kinds of reasons are the major metabolic disorders affecting all age group of people [27]. Chronic alcoholism is one of the many reasons associated with liver injury [28, 29]. Similarly, many allopathic medicines are also known to cause liver damage [30,31,32,33]. Therefore, there is growing need to usage of traditional knowledge of herbal hepatoprotective agents and develop plant-based nontoxic and clinically safe hepatoprotective medicines.

Many herbal extracts are traditionally used as hepatoprotective agents in the ancient Indian and Chinese systems of medicine. The digestion-stimulating effect of Trigonella may emanate from its hepatoprotective role [35]. The phytochemical analysis showed that the *Trachyspermum ammi (L.), Trigonella foenum- greacum L.* and *Piper nigrum L.* plant seed extracts contains a mixture of phytochemicals such as reducing sugars, carbohydrates, proteins, phenolic compounds, flavonoids, steroids, amino acids, saponins, starch, organic, inorganic acids and alkaloids. The qualitative assays indicated that the plant extracts were have potential phyto chemical activity which can be an excellent option to prepare for hepatoprotective, biological and therapautic novel drugs.

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