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PRELIMINARY PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHY OF POLYHERBAL ANTIDIABETIC EXTRACTS

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ABSTRACT

Herbal medicines are used therapeutically all around the world and the use of medicinal plants for management of diseases is probably the oldest existing method that humanity tries to handle the illness. Herbal medicinal products are considered to be free from side effects and less toxic on comparison with synthetic drugs. However, screening of plants for their activities is very essential to evaluate the actual medicinal value of each plant. The plant medicinal value lies in some chemical substances that produce a definite physiological or pharmacological action on the human body such as alkaloids, tannins, flavonoids, saponins etc. Diabetes mellitus has become a serious threat to human life that continues to increase in numbers and its complications impose significant consequences on individuals, families, health systems and countries. Multifactorial metabolic diseases develop several complications like hyperlipidemia, hepatic toxicity, immunodeficiency etc. and hence, instead of mono-drug therapy, the management of the disease requires the combination of herbs that will provide a synergic effect. The present study evaluates the phytochemical constituents of seven anti-diabetic medicinal plants used in traditional medicine by performing chemical tests and thin layer chromatographic studies using various solvent systems. The polyherbal combination of *Phyllanthus emblica (fruit), Phyllanthus amarus (whole plant), Tinospora cordifolia (whole plant), Curcuma longa (Rhizome), Syzygium aromaticum (flower), Piper lognum (fruit)* and *Moringa oleifera (leaf)* are used for the phytochemical evaluation.

KEYWORDS: **Antidiabetic, Polyherbal, Phytochemical, Hydroalcoholic extract, TLC studies, Identification tests.**

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that is characterized by increasing blood sugar level (hyperglycaemia) in the body due to either defective insulin secretion, insulin action or both. The mechanism behind Type 1 diabetes is destruction of pancreatic beta cells resulting in low insulin production and associated with complications like ketoacidosis. As compared to Type 1, Type 2 diabetes is a complex disorder and numbers of factors like abnormal hepatic glucose metabolism, decreased beta-cell function and peripheral insulin resistance are responsible for this disease. In chronic conditions, DM may damage, dysfunction and failure of various organs like kidneys, eyes, nerves, heart and blood vessels.[1] About 108 million people in the world had diabetes in 1980 that was increased to 220 million by 2004 which was further increases to 387 million people in 2014 and estimated to reach 592 million by 2035. If DM is not treated or controlled properly without urgent action, the death rate will increase by more than 50% in the next 10 years.[2,3] The demand for natural products increased in recent times due to its less toxicity and side effects as compared to synthetic drugs available for the treatment. Numbers of studies are carried out in the search of a suitable plant drug since ancient times for treatment of diabetes.[4-6] Globally more than 250,000 to 500,000 species of medicinal plants are available, however small percentage (5-15%) are analytically scrutinized for the presence of natural bioactive phytochemicals[7]. The biological activities of medicinal plants viz antidiabetic, antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic, antimalarial etc are mainly due to the secondary metabolites present in the plants.[8] The quality of phytoconstituents are assessed by preliminary phytochemical screening, chemo- profiling and marker compound analysis using modern analytical techniques. The Thin layer chromatography (TLC) method is an important analytical tool for the separation, identification and estimation of different classes of natural products.[9] In the present study, phytochemical constituents of the individual and polyherbal combination extracts of seven medicinal plants used in traditional medicine for the treatment of diabetes have been evaluated. The extracts of *Phyllanthus emblica (fruit), Phyllanthus amarus (whole plant), Tinospora cordifolia (whole plant), Curcuma longa (Rhizome), Syzygium aromaticum (flower), Piper lognum (fruit)* and *Moringa oleifera (leaf)* have been used for preliminary phytochemical and TLC studies.

MATERIAL AND METHODS

Collection and Authentication of Plant Materials

The Plants were collected from different areas of Thrissur district (Kerala state) in the month of October. The plants were authenticated by Dr. M Kesavan M.S.A.M, (Chief Physician, Amala Ayurvedic Hospital and Research Centre, Amala Nagar, Thrissur, Kerala).

Preparation of extracts[10,11]

The selected part of plants was washed, dried under shade for 20 days and pulverized for further studies. Soxhlet extraction and cold maceration techniques were used for the preparation of plant extracts. The plant powders were used individually for extraction and also the powdered samples (14 g each) were mixed together for preparing the polyherbal extract. All the sample powders (individual and polyherbal) were extracted with three different solvents using Soxhlet apparatus (250 ml of methanol and 250 ml petroleum ether for 5 hours) and water using cold maceration method (intermittent shaking for 12 Hours). Hydroalcoholic (Methanol and water) extract was prepared by extracting with methanol and water in sequence by using Soxhlet extraction method and cold maceration method respectively. All the extracts were filtered through Whatmann filter paper No. 42 (125mm) to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labelled sterile bottles. This activity has been repeated upon requirement of additional samples.

Preliminary Phytochemical screening[12-14]

The chemical tests of the medicinal plants for the screening and identification of bioactive chemical constituents like glycosides, alkaloids, saponins, phenolic compounds, proteins, carbohydrates, flavonoids, and tannins were carried out by using standard procedures.

Tests for glycosides

Legal's test: Appearance of pink to red colour.
Borntrager's test: Ammonia layer acquires pink colour.
Fehling's test: A denser red precipitate.

Test for alkaloids

Dragendorff's test: A red precipitate.
Mayer's test: A creamy-white colored precipitate.
Wagner's test: A reddish-brown precipitate.
Hanger's test: A yellow precipitate.

Test for tannins

Lead Sub-acetate Test: A cream gelatinous or white precipitate.
Ferric Chloride Test: A transient greenish to black/voilet colour.

Test for flavonoids

Aluminium Chloride Test: A yellow precipitate.
Alkaline reagent test: Intense yellow colour which turns to colourless.

Test for Saponins

Emulsion Test: Formation of emulsion.
Frothing Test: Formation of foam or stable froth.

Test for carbohydrates

Molisch's test: Appearance of brown ring at the junction of two liquids.
Iodine test: Blue colour.

Test for phenols

Ferric Chloride test: bluish black colour.

Test for protein
Xanthoproteic test: Yellow colour.
Biuret Test: Violet colour.

Test for Steroids and Terpenoids (chloroform sulphuric acid test)
Steroids: A pink or pinkish-brown.
Terpenoids: A blue, bluish-green.

Thin layer chromatography[15]
The polyherbal extracts (methanol, petroleum ether, water and hydroalcoholic extracts) were subjected to thin layer chromatography (TLC) studies based on conventional one dimensional ascending method using silica gel coated aluminium backed pre-prepared TLC plates (Silica gel 60 F254 plates) as stationary phase. A large number of solvent systems were used as mobile phase for better resolution of the phytochemical constituents and finally one was selected with good resolution and revealing major phytoconstituents. Plate markings were made with soft pencil and the pre-saturation with mobile phase was performed before running the plates. Glass capillaries were used to spot the sample onto the TLC silica plates and studies were performed in TLC chamber. The TLC plates were dried with a blower after completion of run and were visualized under UV light. The active compounds were expressed by the retention factor (R_f) values as calculated by the given formula.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent front}}$$

RESULTS

Phytochemical Analysis
Phytochemical analysis of the individual plant extracts shows the presence or absence of different phytochemical constituents. (Table 1)

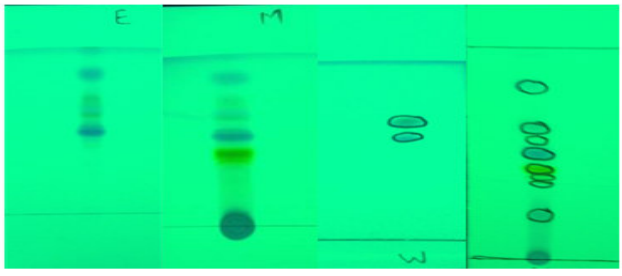
Table 1
Phytochemical Screening of the individual extracts

Phyto Chemical constituents	*Emblica officinalis* (fruit)			*Phyllanthus amarus* (whole plant)			*Tinospora cordifolia* (stem)			*Curcuma longa* (rhizome)			*Syzygium aromaticum* (flower)			*Piper lognum* (fruit)			*Moringa oleifera* (leaves)		
	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
Alkaloids	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-
Glycosides	-	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+
Tannis	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	+	-	+	+	+	-	-	-	+	+	-	-	+	+	+	+	-	+	+
Saponins	+	+	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	+
Steroids	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+
Carbohydrates	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+
Proteins	-	-	+	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-
Phenols	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

E-Petroleum ether extract; M-Methanolic extract; A-Aqueous extract; + present; - absent

Thin layer chromatography
TCL studies were performed for polyherbal extracts based on the individual phytochemical analytical results. The following mobile phases were finalized for TLC studies of different polyherbal extracts under trial and error method based on the resolution achieved.

- Methanol extracts: Toluene: hexane: IPA (6:3:1)
- Petroleum ether extracts: Toluene: Hexane: ethylacetate: IPA (5:3:1:1)
- Water & Hydroalcoholic extracts: Toluene: Hexane: IPA: Water (5:3:1:1)



Petroleum ether Methanol Water Hydroalcoholic

Figure 1
TLC shows the various phytochemical constituents in polyherbal extracts

Based on the TLC profiling obtained for all the four extracts, the R_f Values are calculated and tabulated. (Table 2)

Table 2
Phytochemical Screening of the individual extracts

S.No	Extract Name	No. of spots detected	Rf Value
1	Petroleum Ether	5	0.51, 0.61, 0.73, 0.85, 0.95
2	Methanol	6	0.36, 0.41, 0.52, 0.69, 0.76,0.86
3	Water	2	0.60,0.68
4	Hydroalcoholic	8	0.22,0.37,0.41, 0.45, 0.51, 0.61,0.67, 0.84

DISCUSSION

Herbal remedies provide a popular alternative for treating known and emerging disease and are also in great demand because of their safety, effectiveness and lesser side effects. As per WHO, world's population relied upon traditional medicines for their primary healthcare needs.[16] The rich knowledge in herbal medicines and healthcare leads to intense interest by many pharmaceutical companies for research and development in the quest of discovering novel drugs. However, several medicinal plants are used in the form of crude form for various aspects without scientific evidence of efficacy and hence, at this juncture, the determination of scientific basis for the traditional use of these plants is very important.[17,18] The purpose of extraction procedures for herbal medicinal plants is to attain the therapeutically active portions and to eliminate undesired material by treatment with selective solvents. The extracts may be used as medicinal agent in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as oral solid dosage forms. The phytochemical screening of the individual herbal extracts reveals the presence or absence of certain phytoconstituents such as alkaloids, tannins, carbohydrates, glycosides, proteins, phenols, carbohydrates, steroids, terpenoids and flavonoids. However, saponins, steroids and proteins was absent in many of the extracts. In the present study, the combined phytochemical evaluation of the individual extracts revealed the presence all the phytoconstituents which will have a synergetic effect on the antidiabetic activity. The plants screened for phytochemical constituents appeared to have high potential to act as a source of beneficial drugs and also to improve the health status of patients due to the presence of various compounds that are vital for good health. The use of mixture of solvents in thin layer chromatography (TLC) with variable polarity and ratio will be useful for isolation of pure compound from plant extracts. The selection of appropriate solvent system for particular plant extract can only be accomplished by analyzing the R_f values of compounds in different solvent systems. In the present study, TLC profiling of hydroalcoholic extract shows more number of phytochemicals when compared to other three extracts. Numerous phytochemicals give diverse R_f values in different solvent system and this difference in R_f values of the phytochemicals provides a very significant clue about their polarity and also helps in selection of suitable solvent system for separation of pure compounds using column chromatography. These tests will be useful for qualitative separation and qualification of pharmacologically dynamic medicinal compounds.

CONCLUSION

Herbal antidiabetic medicines are considered to be effective, less toxic and free from side effects when compared to synthetic drugs. In the present study, the combined phytochemical evaluation of the individual extracts revealed the presence of the phyto-constituents such as alkaloids, tannins, carbohydrates, glycosides, proteins, phenols, carbohydrates, steroids, terpenoids and flavonoids which may have a synergetic effect on the antidiabetic activity. TLC profiling of polyherbal hydro-alcoholic extracts show the presence of more number of phytochemicals in comparison to other three extracts and hence can be considered for further studies. Also isolation, identification and characterization of the active compounds will give deeper insight for further developments in the field of antidiabetics treatment.

CONFLICT OF INTEREST

Conflict of interest declared none.

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