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# PHARMACOGNOSTIC STANDARDIZATION AND TLC FINGERPRINTING OF A NOVEL POLYHERBAL ANTIDIABETIC FORMULATION

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## ABSTRACT

India is a rich heritage of medicinal plants and is being used for the treatment of various diseases since centuries ago but there is a lack of scientific standardization to prove the folkloric claims. In recent years there has been an emphasis on standardization of medicinal plants of therapeutic potential. Identification and evaluation of plant drugs by Pharmacognostical study is still more reliable, accurate and inexpensive. The present investigation was evaluation of a poly herbal formulation consisting of eight medicinal plant extracts viz. *Momordica charantia, Gymnema sylvestre, Azadirachta indica, Syzigium cumini, Ocimum sanctum, Picrorrhiza kurroa, Zingiber officinale, Pure Commiphora mukul*. Each extract of polyherbal formulation were showed potent anti-diabetic activity .These were also potent Immunomodulator, antihyperlipidemic, hepatoprotective, Antistress, Expectorant, Anti-inflammatory. Plant material was analysed for various pharmacognostic parameters as per WHO guidelines procedure. Plant materials of formulation were subjected to Physicochemical and thin layer chromatographic study. Formulation was also scanned by UV spectrophotometer over entire UV range of 190to 450 nm for the characterization [1]. Thin layer chromatography of formulation was also developed. Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. As per traditional Indian system of medicines, the combination of herbal drugs is more preferable because it rejuvenates body systems which help to enhance the desired pharmacological activities and considered to be less toxic and free from undesirable side effects than synthetic drugs.

## INTRODUCTION

Diabetes mellitus is a major lifestyle disease with varied etiology and is associated with a variety of irreversible complications. The oral synthetic hypoglycemic agents have been found to have limitations in therapeutic use, primarily because of their side effects.

A study of ancient ayurvedic literature indicates that Prameha means Diabetes was fairly well conceived as a disease entity about 2500 years ago by the vedic physicians. Many indigenous drugs have been in popular use for the treatment of Diabetes since ancient times.

In Ayurveda Diabetes is considered to be one of group of twenty conditions called 'Prameha' in which quality of urine is altered and frequency of micturation increases. 'Sushruta' distinguished two kinds of Madhumehas."Sushruta" states that the patient of the incurable type of Diabetes has a thin, rough body eates little but is very thirsty and is restless and the patients of other type suffer from the 'disease of affluence' which arise in patients who have 'Too much' food, too much leisure, too much comfort etc, oversleeping especially during day, lack of exercise, overeating especially of oily, sweets & fatty (fried) food which increase 'Kapha' are said to cause the disease.

The premonitory symptoms of 'madhumeha' are describes as tartar on the teeth, burning sensation on the palms and the soles of the feet , stickiness of the skin , thirst and a persistant sweet taste in the mouth . The characteristic symptoms also include increased frequency of urine especially during night; delayed healing of wounds and sweetness of urine[1].

The World Health Organization (WHO) released the guidelines to formulate traditional medicine and also provided guidelines for evaluation, safety, and efficacy for public health care[3]. In order to assess the quality control of herbal medicines, standardization of herbal formulations is required which can be achieved using modern sophisticated analytical techniques. As per traditional Indian system of medicines, the combination of herbal drugs is more preferable which enhances the desired pharmacological activities and considered to be less toxic and free from undesirable side effects other than synthetic ones[2].

## MATERIALS AND METHODS

The crude drugs were procured from local market and authenticated by renowned botanist.

**Materials**

| Vernacular name | Botanical name | Parts used |
|---|---|---|
| Kutki | *Picrorrhiza kurroa*[4,5] | Roots |
| Neem | *Azadirachta indica* [4,6] | Leaves |
| Karela | *Momordica charantia*[7-14] | Fruit |
| Jamun | *Syzigium cumini* [15-21] | Seeds |
| Sounth | *Zingiber officinale*[22] | Rhizome |
| Gurmar | *Gymnema sylvestre*[23-28] | Leaves |
| Tulsi | *Ocimum sanctum* [29-34] | Leaves |
| ShudhGuggul | Pure *Commiphora mukul*[35-40] | Resin |

**Thin Layer Chromatography**

The preliminary goal of every analytical investigation is to obtain qualitative information about the sample being tested.

**Selection of Stationary phase**

Separation for pharmaceuticals and drugs can be performed using modified,on-modified and impregnated stationary phases due to a difference in chemical properties between the sorbent material and the compounds of the sample to be separated. For TLC the most frequently used stationary phase is silica Gel[1].

**Stationary Phase**- Precoated silica gel GF plates, Porous surface active silica gels are by far most commonly used sorbents in thin layer chromatography. The skeleton of silica gel is formed from SiO4, 4 –tetrahedrons and displays as amorphous structure. The SIO4 4-tetrahydrons at the surface of the sorbent are saturated by silanol groups (SiOH) or siloxane bridges (Si-S-Si) in adsorption chromatography. The surface silanol groups represent the active centre at which the interaction takes place that are required for the retention of compounds to be separated. These interactions are mainly hydrogen bonding, dipole- dipole interactions and electrostatic interactions. As the retention of sample substances in adsorption chromatography is determined largely by the number of accessible silanolgroups , the strength of the retention is proportional to the specific surface area of the silica gel used[3].

***Selection of the solvent system***

The choice of a mobile phase used in Thin Layer Chromatography is made based on the nature of the solutes to be separated. Either a single solvent or a mixture of solvents can be used depending on the polarity and solubility. Selection of the mobile phase is usually proceeded by consultation of literature sources to find those that were previously used for the separation of the target compounds or similar types of compounds. This followed by a trial and error approach to modify the mobile phase for the particular layer and other local conditions used if necessary. A great variety of factors can be adjusted to achieve the necessary separation[3].

All chemicals and reagents used were Analytical grade.

***Optimization of the mobile phase***

Optimization is based on the analyst's experience and on modification of published data.

However,as the sample composition becomes more complex systematic solvent optimization becomes more important. Poor grade of solvent used in preparing mobile phase have been found to decrease resolution, spot differentiation and reproducibility.

***Selection of Development mode***

Development of a TLC plate is most often carried out in the ascending direction by immersing the lower edge of the plate in the mobile phase in a rectangular glass chamber. In ascending development, the motion of the mobile phase is in upward direction. The plate is introduced in a closed chamber containing mobile phase in such a way that the spot of the sample is above the level of the mobile phase in the chamber. In ascending development, the mobile phase is moving slowly, since its flow is against the force of gravity. Hence this type of a technique is used when there is a little difference in the $R_f$ values of solutes getting separated on the plate.

**Plate Development**: A drop of sample solutions to be analyzed is introduced, with the help of a capillary or a micro syringe, at some point on the planar surface of the stationary phase on a glass plate or on an aluminium foil. Solvent is evaporated from the spots & the plate is placed in the chamber containing a mobile phase (The developer).

The chamber is kept tightly closed so that it becomes saturated with the vapour of the mobile

phase. If the chamber is not saturated, the mobile phase rising through the plate evaporates & then it is not available to the solutes on the plate for the separation purpose. Thus, the proper separation of compounds is not achieved if the chamber is not saturated with the vapour of the mobile phase[3].

**Mechanisms for the separation of Phytoconstituents**

When a substance is applied in dissolved form to the thin layer plate during the separation process, it is transported along by the mobile phase, resides for a certain time on the stationary phase and is then carried along again. In this way the substance is slowed down relative to the velocity of the mobile phase, the more it preferentially resides on the stationary phase the greater the slowing effect will be. Thus substances are also separated which resemble each other in their affinity for the two phase even small differences lead to differences in the chromatographic run. It depends on two basic underlying principles of the differences in affinity: adsorption and partition equilibria. The events can be described physico chemically in the following terms. During adsorption, substances dissolved In the mobile phase are adsorbed on the surface of the sorbent such as silica gel, partitioning results from differences in the solubility in two immiscible phases[3].

***Detection (Visualization ) of spots***

After development, the TLC plate is dried in an open air to evaporate the mobile phase. Compounds are detected on thin layers by their natural color,natural fluorescence under UV light, quenching of Fluorescence or coloured UV absorbing or Fluorescent zones after reaction with an appropriate reagent.

**TLC of Crude drugs of a Polyherbal Formulation (Capsule)**

The methodology of TLC development of crude drugs were shown in Table 1

Table 1 TLC of Crude drug of Polyherbal formulation (Methodology)

| Plant extract | Sample preparation | Stationary Phase | Mobile Phase | Length of Run |
|---|---|---|---|---|
| KARELA | Reflux 2 g of powdered drug with 20 ml of ethanol (95%) for 1 hr. | Silica gel GF 254 | Toluene : Ethyl Acetate : Glacial acetic acid<br>90 : 10 : 0.1 | 115mm |

| | | | | |
|---|---|---|---|---|
| GURMAR | Reflux 2 g of powdered drug with 20 ml of ethanol (95 %) on a hot water bath for | Silica gel GF 254 | Toluene : Ethyl acetate : Glacial Acetic Acid<br>90 : 10 : 0.1 | 115mm |
| NEEM | 2 g of coarsely powdered sample add 20 ml of chloroform and reflux on a hot water bath for 20 minutes. | Silica gel GF 254 | Chloroform : Methanol<br>97 : 3 | 120mm |
| JAMUN | 2 g of powdered drug is refluxed with 20 ml of ethanol (95%) for half an hour and | Silica gel GF 254 | Toluene : Ethyl acetate : Glacial acetic acid<br>90 : 10 : 0.1 | 115mm |
| TULSI | Dissolve 0.1 ml of volatile oil distilled from drug in 5 ml of Acetone. | Silica gel 60 | Toluene : Ethyl Acetate<br>99 : 1 | 8mm |
| KUTKI | Macerate 1 g of powdered drug with 10 ml ethanol with occasional shaking for 30 minute. | Silica gel GF 254 | Ethyl Acetate : Methanol : Glacial Acetic Acid<br>18 : 5 : 0.2 | 120mm |
| SOUNTH | Reflux 1 g of powdered drug with 10 ml dichloromethane. Filter and evaporate the filtrate in 2 ml of dichloromethane. | Silica gel 60 | n-Hexane : Diethylether<br>4 : 6 | 100mm |
| GUGGUL | 2 g of sample + 20 ml ethanol Reflux on water bath for 30 minutes ; | Silica gel G | Petroleum ether : Ethyl Acetate<br>3 : 1 | 120mm |

**UV Scan Test of Crude drugs**

**Sample treatment**

Weighed requiredquantity of powdered crude drug adds 95% ethanol,Shake on a mechanical shaker for 4 hours. Allow to stand for half an hour. Then filter rapidly through a filter and collect the filtrate in a 100 ml volumetric flask. Wash the residue with a little amount of alcohol and add washings to filtrate. Make up the volume to mark with ethanol (95%). Dilute this solution with same solvent to obtain a concentration of about 10 – 20 mg per 100 ml Scan the solution on a UV visible spectrophotometer against ethanol as a blank over a wavelength range of 190 nm to 450 nm.

**Formulation**

Kutki (*Picrorrhiza kurroa*)[4,5]
Extract- 35 mg
Neem( *Azadirachta indica*)[4,6]
Extract- 50 mg
Karela(*Momordica charantia*) [7-14]
Extract- 100 mg
Jamun (*Syzigium cumini*)[15-21]
Extract- 50 mg
Sounth (*Zingiber officinale*)[22]
Extract- 30 mg
Gurmar ( *Gymnema sylvestre*)[23-28]
Extract-50 mg
Tulsi ( *Ocimum sanctum* ) [29-34]
Extract-50mg
Shudh Guggul(Pure*Commiphora mukul*)[35-40]
Extract-35 mg

**TLC of Polyherbal capsule**

**Stationary Phase** - Silica Gel GF 254

**Mobile Phase**- Toluene: Chloroform : Ethyl acetate : GAA (50 : 40 : 10 : 0.1)

**Sample Preparation** - 2 g of capsule content is refluxed with 10 ml of ethanol (95%) for 20 minutes on a hot water bath. Cool and filter the solution. Spot 20 µl of the filtrate.

**Run** - 85 mm

**UV Scanning of Polyherbal capsule**

Transfer about 2.5 g of capsule content to a stoppered conical flask, accurately weighed. Add 75 ml of ethanol (95%) to it. Shake on a mechanical shaker for 4 hours. Allow to stand for half an hour. Then filter rapidly through a filter and collect the filtrate in a 100 ml volumetric flask. Wash the residue with a little amount of alcohol and add washings to filtrate. Make up the volume to mark with ethanol (95%). Dilute this solution with same solvent to obtain a concentration of extract of 0.02g/100ml and 2.5g/100ml of capsule content per 100 ml of ethanol (95%). Scan both the solutions on a UV visible spectrophotometer against ethanol as a blank over a wavelength range of 190 nm to 700 nm

**RESULTS**

1) Macroscopy and UV Scan test of crude drugs

KARELA :-Appearance - The fruit is usually pointed ,ribbed ; bearing numerous triangular tubercles ; giving it the appearance of crocodile's back
Colour– Green, Taste- Bitter, Odour – Characteristic.

GURMAR - Appearance: 2.25 to 5 cm long and 1.2 to 3 cm broad, usually elliptic-ovate to ovate-lanceolate to oboveate leaves.

Colour- Upper surface -dark green, under surface -pale green
Taste - Slightly bitter and astringent, Odour- Characteristic aromatic

NEEM : Appearance : - The leaves are compound, impariopinnate, and the leaflets are 5 – 8 cm long, serrated, lanceolate, acuminate, glabrous,
Colour : - Dark green above and pale beneath, Taste - Bitter, Odour- Characteristic

JAMUN : Appearance : - Rough surfaced , ovoid seeds along with endocarp of fruit; posses two points of attachment
Colour - Brownish black, Taste- Slightly bitter and astringent, Odour- Slight and aromatic.

TULSI : Appearance - Small leaves,
Colour – Green, Taste - Sharp bitter,Odour - Aromatic

KUTKI : Appearance : Aerial stem is very dark brownand wrinkled longitudinally. Upper and lower surface bears a few small root scars, numerous scaly leaves and thins scars.
Colour : Dark brown, Taste : Very bitter.Odour : Slightly unpleasant.

SOUNTH : The rhizomes of Zingiberofficinale branches irregularly. The pieces are 7–15 cm long, 1 –1.5 cm broad and laterally compressed.The branches, known as fingers, arise obliquely from the rhizome; about 1 – 3 cm in length and terminate in depressed scars or in undeveloped buds.
Colour : Buff, Taste : Pungent, Odour : Agreeable and aromatic

GUGGUL: Appearance : The resin occurs as vermicular or stalactite pieces.
Colour : Pale yellow, brown or dull-green, Taste : Bitter, Odour : Balsamic
Results of UV scan test were shown in Table 2.

Table 2 Results of UV Scan Test of crude drugs

| Crude Drug | Concentration | Result (Characteristic peak at ) |
|---|---|---|
| KARELA. | 18.75 mg/100ml | 271, 283 nm |
| GURMAR | 200 mg/100ml | 270, 326, 413 nm |
| NEEM | 9.35 mg/100ml | 221 nm |
| JAMUN | 9.35 mg/100ml | 221 nm |
| TULSI | 9.35 mg/100 ml | 203 nm |
| KUTKI | 4.35 mg/100 ml | 221 nm |
| SOUNTH | 3.75 mg/100 ml | 202 nm |
| GUGGUL | 8.75 mg/100 ml | 219, 327nm. |

2) **UV Scanning of Polyherbal capsule**

A solution containing extract of 2.5 g of the capsule content per 100 ml of ethanol (95%) shows characteristic peaks at about 665 nm, 607 nm, 536 nm. A solution containing extract of 0.02 g of the capsule content per 100 ml of ethanol (95%) shows characteristic peaks at 272 nm

3) TLC of Crude Drugs

Developed spotswere visualizedunder UV 254 nm and derivatization with suitable spray reagent. The results were given in table 3.

| Name of the Crude drug | Visualization | Derivatization with suitable spray reagent |
|---|---|---|
| KARELA | UV 254<br>Spot 1 - Violet colour, Rf- 0.95 | Vanillin sulphuricacid reagent<br>Spot 1 - Blue Colour$R_f$ - 0.20<br>Spot 2 - Blue Colour$R_f$- 0.62 |
| GURMAR | Day Light<br>Spot 1 - Yellow Colour ; Rf - about 0.95<br>Spot 2 - Blue Colour ; Rf - about 0.62<br>Spot 3 - Yellow Colour ; Rf - about 0.52<br>Spot 4 - Greenish blue ; Rf - about 0.39 | Vanillin sulphuricacid reagent<br>Spot 1 - Violet Colour; Rf - about 0.95<br>Spot 2 - Violet Colour; Rf - about 0.43<br>Spot 3 - Blue Colour ; Rf - about 0.29 |
| NEEM : | - | Sulphuric acid (2%) and heat at $105^0$C for 10 minutes<br>Two brownish yellow spots with Rf values about 0.54 and 0.84 are observed. |
| JAMUN | - | Vanillin sulfuric acid reagent.<br>Evaluation - Spot 1 - Violet Pink ; Rf about 0.16<br>Spot 2 - Violet Pink ; Rf about 0.29<br>Spot 3 - Pink ; Rf about 0.7<br>Spot 4 - Blue ; Rf about 0.96 |
| TULSI | - | Vanillin Sulphuric Acid Reagent.<br>Spot 1 - Reddish brown spot - Rf - 0.37 (Eugenol)<br>Spot 2 - Dark Violet spot - Rf - 0.94 (βCaryophylline)<br>Spot 3 - Two Pink spots - Rf - 0.68, 0.45<br>Spot 4 - Violet Spot - Rf- 0.31<br>Spot 5 - Light Blue Spot - Rf - 0.16 |
| KUTKI | UV light (254 nm)<br>Strong fluorescent quenching is visible in both reference and test solution at Rf - 0.61 and 0.46 | - |

| | | |
|---|---|---|
| | corresponding to Picroside I and Kutkoside respectively. | |
| SOUNTH | - | With Barton reagent - Pungent principles appear as prominent blue zones just above the application site and at Rf- 0.2 (Major spot) due to gingerols and / or Shogaols.<br><br>With Vanillin Sulphuric Acid Reagent Prominent blue violet zones at Rf- 0.59 and a major spot near solvent front (Rf - 0.95)due to terpenes |
| GUGGUL | - | Dilute Sulfuric acid<br>Total 7 spots are observed<br>Violet spot ; Rf- 0.091, Violet spot ; Rf- 0.34, Yellow spot ; Rf- 0.46, Violet Spot ; Rf - 0.56, Yellow spot ; Rf - 0.64, Violet spot ; Rf - 0.725, Pink spot ; Rf - 0.86 |
| TLC of Polyherbal capsule | under UV 254 - 5 Violet Spots are observed with Rf values of about 0.89, 0.5, 0.4, 0.34 and 0.28 | With Anisaldeyde sulfuric acid reagent 8 spots are observed . A dark green colour spot with Rf of about 0.9, 5 grey colour spots with Rf values about 0.65, 0.57, 0.47, 0.34 and 0.12. Two blue spots are observed with Rf values of about 0.3 and 0.21 |

## Discussion

TLC is one of the important techniques for the separation of complex mixtures. TLC of Crude drugs of poly herbal formulation were developed using different composition of solvent system and the best resolution and intensity of coloured spots were observed in the above selected solvent systems. UV scanning of crude drugs and poly herbal formulation showed maximum absorbance and characteristic peaks between 202-413 nm. The present study serves the source of literature for isolation and quantitative analysis of herbal drugs to ascertain the purity and potency in formulations.

## Conclusion

Thin layer chromatography is one of the relevant separation technique based on the polarity, solubility and acidity/ basicity of phytoconstituents . The present pharmacognostical study and finger printing of poly herbal formulation yielded a set of qualitative standards that can serve as an important source of information to ascertain the quality and purity of the different plant material composed in this polyherbal formulation.

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