Research Article

Standardisation of Herbal Extract Mixture by HPTLC Method

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ABSTRACT

Herbalism is a traditional medicine or folk medicine practice based on the use of plants and plant extracts. Sometimes the scope of herbal medicine is extended to include fungi, bee products, as well as minerals, shells and certain animal parts. Poly herbal treatment has vital role in diabetes mellitus. Keeping this as an idea a well-known folklore plants *Solanum xanthocarpum* and *Terminalia belerica* were selected for the preparation of herbal extract mixture. For the selected herbs mixture was prepared based on the *in vitro* nitric oxide study. From this 70:30 of *Solanum xanthocarpum and Terminalia belerica* has most effective scavenging activity. So the present study was continued with its Phytochemical analysis, Physico–chemical analysis. The selected herbal extract mixture was further subjected to HPTLC profile.

Keywords: Herbal extract mixture (*Solanum xanthocarpum: Terminalia belerica* - 70:30), Phytochemical analysis, Physico-chemical analysis, HPTLC.

INTRODUCTION

Herbs are prime medicinal agents in traditional and holistic therapies. Particularly in India and China an extensive and intricate herbal science has been developed. An herb is a plant or plant part used for its scent, flavor or therapeutic properties[1]. Herbal medicine products are dietary supplements that people take to improve their health. Many herbs have been used for a long time for claimed health benefits[2].

Currently Phytochemistry have significant development. The technology involves the isolation, extraction, purification and characterization of active constituent from natural origin[2].

The isolated lead compounds are mainly used as therapeutic agent in chronic diseases. Major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value.

MATERIALS AND METHODS

Extraction

Material

The plant specimens for the proposed study were collected from **R. R. Herbals**, Chennai, Tamilnadu.

It was identified and authenticated by **Dr. P. Jayaraman**, Director, Plant Anatomy Research Centre, (PARC) Tambaram, Chennai.

Method

The most useful techniques for general application[3]. All the finely divided solids have the power to adsorb other substance on their surface to a greater or lesser extent.

Thin – layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid.

$$R_f = \frac{Distance\ travelled\ by\ Solute}{Distance\ travelled\ by\ Solvent}$$

The whole plant of *Solanum Xanthocarpum* and the fruits of *Terminalia belerica* were shade dried and coarsely powdered. About 500 gm of the powdered materials were extracted separately by cold maceration procedure successively with solvents of increasing polarity (Petroleum ether (60-80°C), Ethanol and Water). The solvent was filtered and distilled off. Final traces of solvent was removed under vacuum[3]. The yield was noted and it was very high in case of aqueous extract in both herbs.

Phytochemical Test[4]

The preliminary phytochemical screening of petroleum ether, ethanol and aqueous extract of both the plants were carried out for the identification of various phytoconstituents.

The results are tabulated **Table 1.**

Thin Layer Chromatography

Of the various methods of separation and isolation the plant constitutes, the chromatographic procedure originated by Tswett is one of the most useful techniques of general application.

TLC Analysis of Reported Compounds of Solanum xanthocarpum

About 5 g of powdered drug was macerated with 50ml of 2% acetic acid in methanol for 30 min. Then it was refluxed for 90min and filtered. The marc was again macerated as above mentioned procedure. The combined

filtrate was concentrated to about 10 ml and add 1 ml of hydrochloric acid.

The solution was refluxed for 2 hr in boiling water bath, cool and make the solution alkaline with aqueous sodium hydroxide solution (50%) to a pH of 8-9.

Again it was refluxed for 2 hr. Then it was concentrated under reduced pressure to about 5ml.

The concentrated solution was added with 20 ml of water and then it was partitioned with chloroform (2x15ml). The combined chloroform extracts was concentrated and the volume was make up to 10ml[5].

The test sample were spotted on the plate by using fine capillaries. A number of developing solvent system were tried, but satisfactory resolution was obtained in some solvent which are mentioned in the table below.

The result of TLC profile of ***Solanum xanthocarpum*** is given in **Table 2.**

TLC Analysis of Reported Compounds of Terminalia belerica

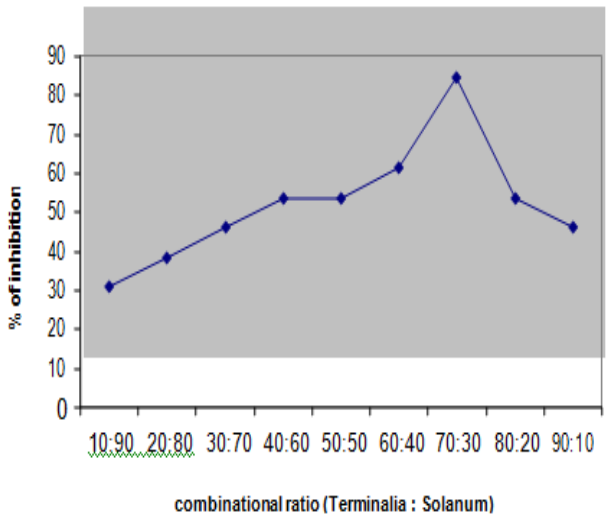
About 0.4 gm powdered drug was refluxed with methanol (50ml) for 30 minutes. Then the solution was cooled and filtered. The filtrate was evaporated to dryness.

After this the residue was dissolved in methanol and the solution was make up to 50 ml[6].

The test sample were spotted on the plate by using fine capillaries. A number of developing solvent system were tried, but satisfactory resolution was obtained in some solvent which are mentioned in the Table 3.

Preparation of Herbal Extract Mixture

Preparation of herbal extract mixture was mainly based on the preliminary phytochemical test & *in vitro* antioxidant activity by Nitric oxide scavenging method. Petroleum ether, ethanol and water extract of both the selected plants (*Terminalia belerica* and *Solanum xanthocarpum*) were subjected to preliminary phytochemical test. The phytochemically active extract of the plants has been selected.



Standardisation of Herbal Extract Mixture

Standardisation of herbal extract is a measure of determination of identification, quality and purity. There are various methods used to determine the efficacy of the drug. So, in the present study herbal extract mixture was subjected to physicochemical evaluation, phytochemical estimation, fluorescence analysis, TLC and HPTLC study, which are helpful for the identification of crude drug and phytoconstituents of drug material.[4&5]

Air dried coarsely powdered drugs of *Terminalia belerica* and *Solanum xanthocarpum* were mixed in the ratio of 70:30 and subjected to the following analysis.

Physico Chemical Analysis

The selected herbal extract mixture was subjected to Physico-chemical analysis such as

Ash Values & Extractive Values[7]

Loss on Drying & Foam Index[8]

Swelling Index[9]

The results of Physicochemical analysis are reported in **Table 4.**

Phytochemical Estimation

Fat Content[9]

Resin Content[9]

Bitterness Content[9]

Total Alkaloidal Content[9]

Tannin Content[10]

Total Phenolic Content[11]

The results of Phytochemical estimation were tabulated in **Table 5.**

Fluorescence Analysis[11]

The results of fluorescence analysis are given in **Table 6.**

High Performance Thin Layer Chromatography

Now a days HPTLC is a very important versatile separation technique in the standardisation of herbal of extract[13,14].

It is mainly used for the isolation and identification of herbal extract and its constituents.

So, in the present study an attempt was taken to separate and identify the phytocomponents of herbal extract mixture and it was compared with individual extract.

Development of Chromatogram

A rectangle twin trough glass chamber was used in the experiment.

To avoid insufficient chamber saturation and the undesirable edge effect, a smooth filter paper was placed in the glass chamber and was placed in the glass chamber and was allowed to be soaked in the developing solvent[15].

Procedure

The plate was dipped in a saturated chromatographic chamber containing the solvent system and was allowed to elute up to 8 cm and was air dried[16,17].

The result for HPTLC analysis of *Terminalia belerica* & Herbal extract mixture were shown in figures 1-4.

RESULTS

The selected herbal extract was analysed for its phytochemical investigation and its results are tabulated below.

Table 1: Phytochemical Test

S. No.	Test	*Solanum xanthocarpum*			*Terminalia belerica*		
		Pet	Eth	Aqu	Pet	Eth	Aqu
1.	**Alkaloid**						
	a) Mayer's test	-	+	+	-	-	-
	b) Dragendorff's test	-	+	+	-	-	-
	c) Hager's test	-	+	+	-	-	-
	d) Wagner's test	-	+	+	-	-	-
2.	**Glycosides**						
	Anthrone test	-	+	+	-	+	+
3.	**Cardiac glycoside**						
	Kellar killani test	-	+	+	-	+	+
4.	**Anthroquinone test**						
	a) Borntrager's test	-	-	-	-	-	-
	b) Legal test	-	-	-	-	-	-
	c) Baljet test	-	-	-	-	-	-
5.	**Carbohydrate**						
	a) Molish's test	-	-	+	-	+	+
	b) Barfod's test	-	-	+	-	+	+
	c) Fehling's	-	-	+	-	+	+
	d) Benedict's test	-	-	+	-	+	+
6.	**Protein**						
	a) Millon's test	-	-	+	-	+	+
	b) Biuret test	-	-	+	-	+	+
	c) Xanthoprotein test	-	-	+	-	+	+
7.	**Amino acid**						
	Ninhydrin test	-	-	-	-	-	-
8.	**Steroids**						
	a) Libermann's test	+	-	-	+	-	-
	b) salkowski test	+	-	-	+	-	-
9.	**Flavonoids**						
	Shinoda test	-	-	-	-	-	-

Aqu- aqueous extract, eth-ethanol, pet – petroleum ether, + present,- absent

Table 2: Thin Layer Chromatography of *Solanum xanthocarpum*

S. No.	Mobile phase	Detecting agent	Colour of spot	R_f value
1.	Ethyl acetate: Toluene: glacial acetic acid (7.5 : 2.0 : 0.5 : 0.2)	Alcoholic ferric chloride	Blue	0.76
2.	Toluene : Ethyl acetate: glacial acetic acid: Formic acid (20 : 45 : 20 : 5)	Anisaldehyde in sulphuric acid	Blue	0.82

Table 3: Thin Layer Chromatography of *Terminalia belerica*

S. No.	Mobile phase	Detecting agent	Colour of spot	R_f value
1.	Ethyl acetate : methanol : Water (8:1:0.8)	Dragendroff's Reagent	Orange	0.71
2.	Chloroform : Acetone : Formic acid (7.5:1.65:0.85)	Dragendroff's Reagent	Orange	0.34

Table 4: Physicochemical Parameters of Herbal Extract Mixture

S. No.	Parameters	Results
1.	**Ash value**	
	• Total ash	8.0 % w/w
	• Acid insoluble ash	2.3 % w/w
	• Water soluble ash	5.4 % w/w
2.	**Extractive value**	
	➢ Water soluble	5.8 % w/w
	➢ Alocohol soluble	4.8 % w/w
	➢ Pet. eth soluble	0.8 % w/w
3.	**Loss on drying**	7.8 % w/w
4.	**Swelling index**	0.2 ml
5.	**Foam index**	1.2 cm

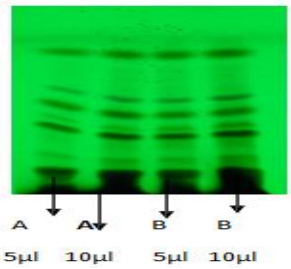
Table 5: Phytochemical Estimation of Herbal Extract Mixture

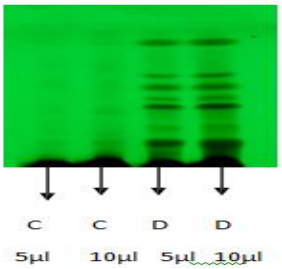
S. No.	Parameters	Results
1.	Fat content	5.5 % w/w
2.	Resin content	0.6 % w/w
3.	Bitterness value	12.83
4.	Total alkaloidal content	9.6 % w/v
5.	Total tannins	6.98 % w/w
6.	Total phenolic content	8.75 % w/w

Table 6: Fluorescence Analysis of Herbal Extract Mixture

S. No.	Reagent	Daylight	UV light (254 nm)
1.	Methanol	Brown	Green
2.	Water	Yellow	Dark green
3.	50% Hcl	Yellow	Green
4.	1% Hcl	Light yellow dark	Greenish yellow
5.	50% H_2SO_4	Brown Yellowish	Reddish black
6.	1N NaOH	Orange Dark	Yellowish green
7.	Alcoholic KOH	Reddish Black	Reddish black
8.	10% NaOH	Dark yellowish Brown	Dark greenish yellow

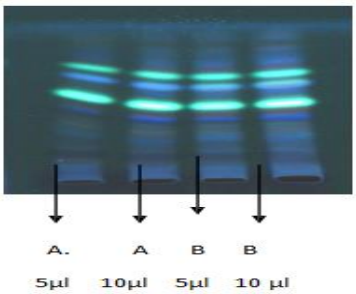
HPTLC of Aqueous extract of Solanum xanthocarpum and Herbal Extract Mixture (5µl at 254nm)

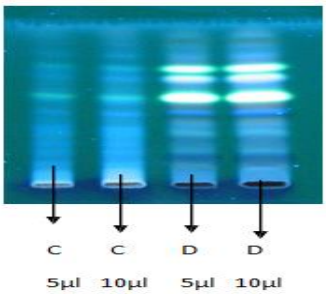




A. *Terminalia belerica*
B. Herbal extract mixture
C. *Solanum xanthocarpum*
D. Herbal extract mixture

HPTLC of Aqueous extract of Solanum xanthocarpum and Herbal Extract Mixture (5µl at 366nm)





A. *Terminalia belerica*
B. Herbal extract mixture
C. *Solanum xanthocarpum*
D. Herbal extract mixture

DISCUSSION

In Ayurvedic medicine *Terminalia belerica* and *Solanum xanthocarpum* have been used in various ailments[18]. The present study was aimed to synergetic activity of herbal extract mixture.

Results of phytochemical test revealed that aqueous extract of *Solanum xanthocarpum* showed positive test for alkaloids, cardiac glycoside, tannin, protein, carbohydrate and triterpenoids. Where as aqueous extract of *Terminalia belerica* showed cardiac glycoside, tannin, protein and triterpenoids. Result of TLC analysis revealed the presence of alkaloids in the *Solanum xanthocarpum* and tannin in *Terminalia belerica*.

Herbal extract mixture was prepared in different combination (aqueous extract of *Terminalia belerica* : aqueous extract of *Solanum xanthocarpum*).

The ratio 70:30 (*Terminalia belerica* : *Solanum xanthocarpum*) showed maximum free radical scavenging property. So, it was selected for further pharmacological activity and it was standardised by physicochemical analysis, Phytochemical estimation, TLC and HPTLC profile.

SUMMARY AND CONCLUSION

In folk medicine they are effective either single (or) combinational therapy. Based on ethnopharmacological sources, the potent hypoglycemic herbs of Indian origin *Solanum xanthocarpum* and *Terminalia belerica* were selected for the present work.

The plants were extracted with solvents of increasing polarity. The herbal extract was standardised by physicochemical analysis.

The results provide a protocol for identification and authentification of drugs in the herbal extract mixture and standardisation of bioactive constituents.

The presented finger print and chromatogram of HPTLC analysis of herbal extract mixture provided a data for identification and standardisation of bioactive constituents.

In conclusion, this present work supports the earlier claims of plant for the treatment of diabetes.

This study also provides evidence that aqueous extract of plants *Terminalia belerica : Solanum xanthocarpum* (70:30) have both physical and Phytochemical data are proof for standardization of herbal extract mixture.

REFERENCES

1. Acosta M.C, Bernardello. Karyotype analysis in several south American species of solanum and *Lycianthes rantonnei* (Solanaceae). *Taxon,* Vol 54(3), 2005, 65-71.
2. Asima Chaterjee, Dr. Satyesh Chandra Pakrashi. "The Treatise on Indian Medicinal Plant", National Institute of Science Communication, 1992, CSIR, New Delhi.

3. Kotate. Text book of Pharmacognosy, Published by Nirali Prakashan15th edition, 1994, 97-132.
4. Kotate. Text book of Pharmacognosy, Published by Nirali Prakashan 17th edition, 1997, 65-74.
5. Kusano. Analysis of Feeding Patterns of Albino Rats on Powder Bait Norbormidei, Japanese Society of Applied Entomology and Zoology, 569–574.
6. Chandrashekhar. Hypoglycemic activity of *Nymphaea stellata* leaves ethanolic extract in alloxan induced diabetic rats, *Fitoterpia* 78, 2008, 45-59.
7. Anonymous, Wealth of India, Publication Information Directorate CSIR New Delhi, vol.1, 1985, 74, 2(A) – 53.
8. Ansari H. "Essentials of Pharmacology'' 1st edition, 2005, Birla Publication Pvt.Ltd, 1-25.
9. Murkujee, Text book of Pharmacognosy, CBS Publisher, 2002, 123–131.
10. Asima Chaterjee, Dr. Satyesh ChandraPakrashi. "The Treatise on Indian Medicinal Plant'', National Institute of Science Page Page 14 of 14 Communication, CSIR, new Delhi, Vol 2, 1992, 55.
11. Kar. Studies on hypoglycaemic activity of *Solanum xanthocarpum* Schrad. & Wendl. Fruit extract in rats journal of Ethnopharmacology, Volume 108, Issue 2, 2006, 24 November, 251-256.
12. Chase and Prat. A report on the quality control parameters of aerial parts of *Pluchealanceolata* (DC.) Oliv. & Hiern, Asteraceae, Farmacogn. Vol. 20 No. 4, 1949, 27-34.
13. Chase CR, Prat RJ, J.Am. Pharm. Assocition Science, 38th edition, Vol 1, 1949, 449-750.
14. Chopra R.N, S.L. Nayar, I.C. Chopra. Glossary of Indian Medical Plants, 4th edition, CSIR Publisher and Distributor, 1988, New Delhi, 230, 241.
15. Colobel and Heber Drury. The Useful plants of India, CBS Publisher and Distributor, Delhi, 1991, 397, 417-418.
16. Donald J Ecobichon. "The Basis Of Toxicity'', 2nd edition CRC Press, 1997, New York, pg 43-49.
17. Dutt U.C. The Materia Medical of the Hindus, CBS Publisher and Distributor, Delhi, 1986, 208-209, 162-164.
18. Dwivedi. The management of asthma with single herbs and vasadikwath- an Ayurvedic preparation, Biotechnological Developments of Herbal Medicine" NBRI, Lucknow, UP, 2003, India p.165.

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