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Comparative Qualitative Phytochemical Analysis of the Different Parts of *Tinospora crispa*: A Contribution to Sustainable Use of the Plant Species

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ABSTRACT

The plant screening for phytochemical constituents seems to have the potential to act as a source of useful drugs and cures many infections as a result of the presence of various bioactive compounds that evident to have enormous activity against array human pathogens. The objective of the study was to undertake a comparative qualitative phytochemical analysis of the different parts of *Tinospora crispa* (*T. crispa*), a traditional herb used against several diseases. The different parts of *T. crispa* were extracted using maceration method. The results of phytochemical screening indicated that *T. crispa* contains alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. The TLC profiles of samples, depicted through the *Rf* values of resolved compound bands and the solvent system selected for the best results of TLC was Toluene: Ethyl acetate: Formic acid (7:5:1) and (5:4:1) for gallic acid and quercetin respectively. The study will provide referential information for the correct identification of the bioactive compounds and a suitable solvent system for separation of those compounds from the *T. crispa*. These findings suggested that *T. crispa* leaves and flower extract could be a potential source of drugs which in future may serve for the production of synthetically improved therapeutic agents.

Keywords: *Tinospora crispa,* Phytochemical screening, TLC profiles, Gallic acid, Quercetin

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INTRODUCTION

Over three-quarters of the world population relies mainly on plants and plant extracts for health care and more than 30% of the entire plant species, at one time or the other was used for medicinal purposes [1]. The use of plants for medicinal purpose is probably as old as the history of mankind and their uses in the industrialized societies have led to the extraction and development of several drugs from as well as from traditionally used folk medicine. Extraction and characterization of several active phytochemicals from these green factories have given birth to some high activity profile drugs [2]. It has been estimated that in developed countries such as United States, plant-drived drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is recognized in all countries over the world and these provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine [3]. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (phytochemicals) [4]. Development of drugs based on natural products has had a long history in the US, and in 1991, almost half of the best selling drugs were natural products or derivatives of natural products [5]. Natural products are chemical compounds derived from living plants or animals. Drugs derived from natural products are usually secondary metabolites and their derivatives Comparison of the phytochemical composition of different plant parts may lead to the utilization of plants parts, in particular the aerial parts, with minimum adversity to the conservation of the plants. In a study by Srivastava et al [6], the major bioactive constituents of the different parts (roots, stem and flowers) of *Taraxacum officinale* were analysed for comparison. Saponins, flavonoids, alkaloids, phenols were highly concentrated in the stems, roots and flowers, with higher concentration of flavonoids in the flower extract. In another separate study, the phytochemical composition of the root and leaf parts of the medicinal herb, *Hypochaeris radicata L.* were also investigated [7]. The findings of the study showed that alkaloids, cardiac glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids were found in both the leaf and root extracts. *T. crispa* (Menispermaceae) a climber plant found in tropical and subtropical India and parts of the Far East (such as Indonesia, Malaysia, Thailand and Vietnam), and in primary rainforest or mixed deciduous forest [8,9]. The plant has been recently showing an ethnopharmaceutical uses for the treatmentof fever,

diabetes, hypertension, cholera, rheumatism, hyperglycemia, wounds, intestinal worms, and skin infections.Besides that, *T. crispa* is also used to treat tooth and stomachaches, coughs, asthma and pleurisy [10-14]. It was revealed that the chemical constituents isolated from various parts of *T. crispa* contained flavonoid and quaternary alkaloids including flavavone-O-glycosides (apigenin), berberine, picroretoside, palmatine [15, 16], borapetol Aand B, borapetoside A and B, tinocrisposide, N-formylanondine, N-formylnornuciferine, N-acetyl nornuciferine, c-sitosterol, picroretine and tinotubride [17]. Two newtriperpenes, cycloeucalenol and cycloeucalenone from *T. crispa* were previously isolated [18]. In this regard, the primary aim of the study was to undertake the qualitative phytochemical analysis of different parts (flowers and leaves) of *T. crispa* with intention of motivation for usage of plant parts with less adverse implications for the survival of the plant species.

MATERIALS AND METHODS

Plant material

The plant *Tinospora crispa* (leaves and flower) was collected from local area of Bhopal (M.P.) in the month of July, 2019. The leaves and flower plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf was air dried under room temperature. The dried plant samples were cut and grinded to make it in powder form.The powdered samples were stored in clean, dry and sterile container for further use.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine- Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Quercetin and gallic acid was kindly provided by Scan Research Laboratories, Bhopal (India).

Extraction procedure

The shade dried material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 100gm of dried plant material were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 80: 20) using maceration method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [19].

Qualitative phytochemical analysis of plant extract

The *T. crispa* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [20, 21]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Thin layer chromatography

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid solvent system used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples.

RESULTS AND DISCUSSIONS

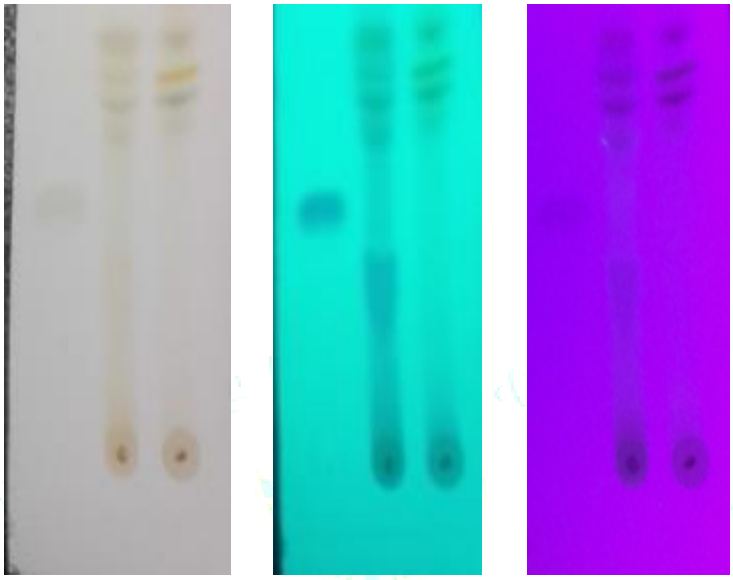
The results of qualitative phytochemical analysis of *T. crispa* extracts were presented in Table 1. The results revealed the presence of flavonoids, phenol, carbohydrate and saponins in leave and flower extract. There was absence of alkaloids, glycosides and protein, amino acid in leave and flower extract. The result of thin layer chromatography of *T. crispa* extracts is presented in table 2 and Fig. 1 & 2. The TLC studies of the hydroalcoholic extract of *T. crispa* with solvent system Toluene: Ethyl acetate: Formic acid in ratio of (7:5:1) and (5:4:1) for gallic acid and quercetin with Rf values was found 0.68 and 0.645 respectively.

Table 1: Result of Phytochemical screening of hydroalcoholic extract of *Tinospora crispa*

S. No.	Constituents	Leaves extract	Flower extract
1.	**Alkaloids** Wagner's Test:	-ve	- ve
2.	**Glycosides** Legal's Test:	- ve	- ve
3.	**Flavonoids** Alkaline Reagent Test: Lead acetate Test:	+ ve - ve	+ ve + ve
4.	**Diterpenes** Copper acetate Test:	+ ve	- ve
5.	**Phenol** Ferric Chloride Test:	+ ve	+ ve
6.	**Proteins & Amino Acids** Xanthoproteic Test:	- ve	- ve
7.	**Carbohydrate** Fehling's Test:	+ ve	+ ve
8.	**Saponins** Froth Test:	+ ve	+ ve

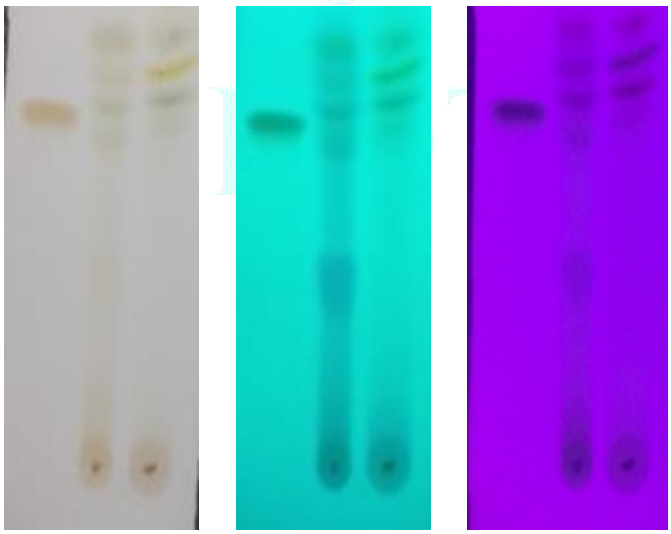
Table 2: Calculation of R_f Value of hydroalcoholic extract of *T. crispa*

S. No.	Compound	Mobile Phase	Rf Value
1.	Gallic acid	Toluene: Ethyl acetate: Formic acid (7:5:1)	0.68
2.	Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.645



Normal Light Short U.V Long U.V

Figure 1: Spot-1 Gallic acid, Spot-2 Leaves extract of *T. crispa*, Spot -3 Flower extract of *T. crispa*



Normal Light Short U.V Long U.V

Figure 2: Spot-1 Quercetin, Spot-2 Leaves extract of *T. crispa*, Spot -3 Flower extract of *T. crispa*

CONCLUSION

Qualitative phytochemical analysis of plant parts extracts is important as it indicate the nature of phytochemicals that are possessed by such medicinal plants. The results of the current study suggest more similarities in the phytochemical compositions of the different parts of *T. crispa* which is likely to contribute to some similarities in their biological activities. Thus, the substitution of the roots of *T. crispa* with the aerial parts could be encouraged as contribution to the sustainable usage of the plant species in traditional medicine.

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