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ESTIMATION OF TOTAL PHENOL, FLAVONOIDS AND ALKALOID CONTENT AND EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *TINOSPORA CRISPA* LEAVES AND FLOWER EXTRACTS

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ABSTRACT

Antioxidant and antimicrobial activity of a hydroalcoholic extract of *Tinospora crispa* leaves and flower were evaluated in the current study. Total phenol, flavonoids and alkaloid content was estimated following standard procedures. Antioxidant activity was assessed by using 2, 2- diphenyl-1-picryl-hydrazyl (DPPH) assay using ascorbic acid as standard antioxidant and antimicrobial activity was calculated by the disc diffusion method. The total phenolic content in leaves and flower extract was found to be 3.600 and 2.044 in mg/g equivalent to gallic acid. The total flavonoid content in leaves and flower extract was found to be 2.491 mg/g and 1.258 mg/g, respectively, equivalent to quercetin. The total alkaloid content in leaves and flower extract was found to be 3.228 mg/g and 0.847 mg/g, respectively, in equivalent to atropine. IC_{50} for standard ascorbic acid was found to be 17.68µg/ml and for hydroalcoholic extract of leaves and flower was found to be 57.17µg/ml and 94.13 µg/ml, respectively. Antimicrobial activity results suggest that *Tinospora crispa* is a potential source of broad-spectrum antimicrobial agents. *Tinospora crispa* leaves and flowers is potential sources of antioxidant and antimicrobial agents and could be used as a natural antioxidant.

Keywords: *Tinospora crispa, Total* phenol content, Total flavonoids content, Total alkaloid content, Antioxidant activity, Antimicrobial activity.

1. INTRODUCTION

Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Natural plant products have been used for therapeutic purposes since time immemorial, and their use is of greater demand nowadays. Majority of the users rely on herbal medicines for health care because the other treatment options are more expensive and are often thought to be more associated with serious side effects [1]. Therefore, there is continuous and urgent need to discover new therapeutic compounds with diverse chemical structures and also novel mechanism of action is required for new and emerging infectious diseases [2]. Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, and free radicals, play a crucial role in the development of various ailments such as immune depression, diabetes mellitus, ageing, dementia, carcinoma, and Parkinson's disease [3]. Many natural herbs contain antioxidant compounds which protects the cells against the damaging effects of ROS. Though our body is safeguarded by the natural antioxidant defense, there is always a demand for antioxidants from external natural source. In addition, secondary metabolites, such as phenolic compounds, flavonoids, alkaloids, and tannins are widely distributed in plants and are reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic effect, etc. [4]. *Tinospora crispa* (Willd.) Miers ex Hook. F. & Thoms (syn *T. cordifolia*) is a climber found in South East Asia and in tropical India. It naturally occurs in primary rainforests or mixed deciduous forests up to 1,000 m above sea level [5]. This plant is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. South East Asian folk medicine considers *Tinospora crispa* as universal medicine since it had long been used in the alleviation of various health conditions. *Tinospora crispa* is also a common component of many conventional Indian herbal preparations. It is used as a tonic, antispasmodic, anti-inflammatory, antiarthritic, antiallergenic, and antidiabetic [6].

Thus, this particular plant species *Tinospora crispa* is available in abundant and can be utilized to generate novel medicinal compounds to cure emerging diseases.

Hence, the present study was aimed at exploring the positive medicinal values of *Tinospora crispa* by evaluating the antioxidant activity, relative content of total phenol, flavonoids, alkaloids in leaves and flower extracts.

2. MATERIAL AND METHODS

2.1. Plant material collection

The leaves and flowers of *Tinospora crispa* were collected from local area of Bhopal (M.P.) in the month of Nov 2020 to Jan, 2021. Drying of leaves and flowers was carried out under the shade. Dried *Tinospora crispa* leaves and flowers were preserved in plastic bags and closed tightly and powdered as per the requirements.

2.2. Defatting and extraction of plant material

Plant materials were extracted in hydroalcoholic solvent (ethanol: water: 80: 20) by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis.

2.3. Determination of total phenolic content

The total phenolic content (TPC) of *Tinospora crispa* leaves and flower extracts were determined by the modified Folin-Ciocalteu method [7]. For preparation of standard gallic acid solution, 10 mg gallic acid was weighed, transferred to a volumetric flask and added 10 ml methanol to dissolve gallic acid. The stock solution was diluted in methanol to give working standard of (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25µg/ml). For plant extract solution preparation, weighed 10 mg of plant extract and dissolved in 5 ml methanol, filtered the solution and adjusted the volume upto 10 ml. Firstly, taken 2 ml of solution of selected plant extracts or standard in 10 ml volumetric flask separately. Added 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 (v/v)) and one ml of sodium carbonate to each flask. Subsequently, reaction mixture was vortexed for 15 seconds and left for 15 minutes at room temperature for color development. After incubation of reaction mixure, absorbance at 765 nm using a spectrophotometer was recorded [7].

2.4. Total flavonoid content

Determination of total flavonoid content (TFC) of *Tinospora crispa* leaves and flower extracts were based on Aluminium chloride method [8]. For preparation of standard quercetin solution, dissolved 10 mg quercetin in 10 ml methanol. Diluted the stock solution in methanol to give working standard of (5µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml). For preparation of plant extract solution, taken 10 mg plant extracts and dissolved into 5 ml of methanol, filtered and adjusted the volume upto 10 ml with methanol. For estimation of total flavonoid contents (TFC) in plant samples, added 1 ml of 2% $AlCl_3$ methanolic solution into 3 ml of different dilutions of standard solution of quercetin (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25µg/ml) or plant extract and left for 15 minutes at room temperature. Subsequently, the solution was mixed well and the absorbance was recorded against a freshly prepared blank reagent at 420 nm using spectrophotometer [8].

2.5. Estimation of total alkaloids content

For preparation of standard atropine solution, dissolved 10 mg atropine in 10 ml methanol. Diluted the stock solution in methanol to give working standard of (40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml, 120 µg/ml). For preparation of plant extract solution, taken 10 mg plant extracts and dissolved into 5 ml of methanol, filtered the solution and adjusted the volume upto 10 ml with methanol. The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. The solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer [9].

2.6. Antioxidant activity

The DPPH radical scavenging activity of leaves and flower extracts was evaluated by the method described by Lee JY *et al.,* 2004 [9] with slight modification. Ascorbic acid (10-100 µg/ml) was used as the standard. Plant extract (1.5 ml) at different concentrations (10-100 µg/ml) were treated with 1.5 ml of 0.2 mmol DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol solution. The reaction mixture was incubated in the dark at room temperature for 30 min. The absorbance

of the sample and standards was measured at 517 nm. The ability of the plant extract and standard to scavenge the DPPH radical was calculated as percentage inhibition of absorbance by using the following formula and IC_{50} values were determined.

Calculation of % reduction = {(Control Absorbance - Test absorbance)/ Control Absorbance} X 100

2.7. Antimicrobial activity

Diameter of zone of inhibition was determined using the paper disc diffusion method [10, 11]. A swab of the bacteria suspension containing 1×10^8 cfu/ml was spread on to Petri plates containing nutrient agar media. Each extract was dissolved in ethanol to final concentration of 10 mg/ml. Sterile filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37°C for 24 h. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded. The data is expressed as mean ± Standard Deviation (SD).

3. RESULTS AND DISCUSSION

The activity of any kind of phytomedicine is principally depending upon the presense of phytochemicals. In this study, we have quantified the presence of total phenolic, flavonoids and alkaloid content in the leaves and flower extract of *Tinospora crispa* was observed. The observed absorbance is tabulated in table 1-3.

Total phenolic compounds (TPC) was expressed as mg/ 100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

The total phenolic content is expressed as mg gallic acid equivalent (GAE) per gram of sample (mg GAE/g). The total phenolic content in leaves and flower extract was found to be 3.600 and 2.044 in mg/g equivalent to gallic acid.

Table 1: Results of estimation of total phenolic, flavonoids & alkaloid content of hydroalcoholic extract of *Tinospora crispa*

S. No	Extracts	Total phenol content (mg/100 mg of dried extract)	Total flavonoids content (mg/100 mg of dried extract)	Total alkaloid content (mg/100 mg of dried extract)
1	Leaves	3.600	2.491	3.228
2	Flower	2.044	1.258	0.847

Table 2: % Inhibition of ascorbic acid and hydroalcoholic extract of *Tinospora crispa* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition		
		Ascorbic acid	Leaves extract	Flower extract
1	10	44.65±4.4	30.14±2.1	24.68±1.9
2	20	48.62±3.2	36.47±3.2	30.14±2.3
3	40	65.34±3.4	45.77±4.8	35.98±3.5
4	60	69.65±2.9	53.93±3.7	41.55±5.1
5	80	77.41±1.3	58.25±2.8	46.87±2.6
6	100	84.13±3.2	63.45±3.1	50.14±3.2
	IC_{50}	17.68±4.1	57.17±2.3	94.13±1.1

Total flavonoid content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.032X+0.018$, $R^2=0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance.

The total flavonoid content is expressed as mg quercitin equivalent (QE) per gram of sample (mg QE/g). The total flavonoid content in leaves and flower extract was found to be 2.491 and 1.258 in mg/g equivalent to quercetin.

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $Y=0.007X+ 0.024$, $R^2=0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance.

The total alkaloid content is expressed as mg atropine

equivalent (AE) per gram of sample (mg AE/g). The total alkaloid content in leaves and flower extract was found to be 3.228 and 0.847 in mg/g equivalent to atropine.

DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the *in vitro* antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm. IC_{50} for standard ascorbic acid was found to be 17.68μg/ml and for hydroalcoholic extract of leaves and flower was found to be 57.17 μg/ml and 94.13 μg/ml, respectively. In order to study the effects of these compounds on biological system more studies are needed as these compounds might be responsible for use of this plant in different diseases [12].

In this work, the antimicrobial effects of hydroalcoholic leaves and flower extract of *Tinospora crispa* were studied using disc diffusion method. The results tabulated in table 5 showed different sensitivity levels for the tested strains *Streptococcus mutans, Bacillus subtilis, Staphylococcus aureus, Salmonella bongori, Klebsiella pneumonia, Escherichia coli, Aspergillus niger, Candida albicans* and *Aspergillus flav*.

Table 3: Results of sensitivity of leaves and flower of hydroalcoholic extract of *Tinospora crispa*

S. No.	Microbes Codes	Microbes	Leaves extract	Flower extract
1.	Bact-1	*Streptococcus mutans*	No	No
2.	Bact-2	*Bacillus subtilis*	No	Yes
3.	Bact-3	*Staphylococcus aureus*	No	No
4.	Bact-4	*Salmonella bongori*	Yes	Yes
5.	Bact-5	*Klebsiella pneumoniae*	Yes	Yes
6.	Bact-6	*Escherichia coli*	No	Yes
7.	Fungus-1	*Aspergillus niger*	No	No
8.	Fungus-2	*Candida albicans*	Yes	No
9.	Fungus-3	*Aspergillus flavus*	No	No

Table 4: Antimicrobial activity of hydroalcoholic extract of *Tinospora crispa* against selected microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		Hydroalcoholic extract of leaves extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Salmonella bongori*	8±0.5	9±0.57	10±0.74
2.	*Klebsiella pneumoniae*	6±0.74	6±0.5	7±0.57
3.	*Candida albicans*	7±0.5	8±0.74	9±0.74
		Hydroalcoholic extract of flower extract		
1.	*Bacillus subtilis*	8±0.47	9±0.47	10±0.47
2.	*Salmonella bongori*	8±0.47	9±0.47	13±0
3.	*Klebsiella pneumoniae*	15±0.47	19±0.47	25±94
4.	*Escherichia coli*	10±0.47	12±0.47	14±0.47

Antimicrobial activity was performed against strains of human pathogenic bacteria by well diffusion method. In disc diffusion, hydroalcoholic leaves and flower extract of *Tinospora crispa* showed good antimicrobial activity against gram positive bacteria. These results suggest that *Tinospora crispa* is a potential source of broad-spectrum antimicrobial agents. The antimicrobial activity of the leaves and flower extract may be attributed to the high content of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes [13]. Moreover, flavonoids are synthesized by plants in response to microbial infection. Phenolic compounds with a C3 side chain at a lower level of oxidation and containing no oxygen have often been reported to be antimicrobials [14]. The partially hydrophobic nature of their phenolic compounds has also been reported to be responsible for their antimicrobial activity. The mechanism of the toxicity of polyphenols against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesins, cell envelope transport proteins and non-specific

interactions with carbohydrates [15]. The antifungal and antimicrobial activity of phenolic and flavonoid compounds has been reported previously [16-18]. Isolation of the responsible elements is necessary for fully elucidating the antibacterial activity of these crude extracts. This might also provide insight about their possible use in food and non-food systems.

4. CONCLUSION

Estimation is the foundation which provides prospect to concret the use of certain traditional values. From the current study, we can conclude that hydroalcoholic extract of *Tinospora crispa* leaves and flowers have a significant amount of secondary metabolites. Our results suggest that *Tinospora crispa* leaves and flowers is a potential source of antioxidant and antimicrobial agents and could be used as a natural antioxidant and preservative in food and non-food systems.

Conflict of Interests

All authors declare no conflict of interest.

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