COMPARATIVE PHYTOCHEMICAL ANALYSIS & ANTIOXIDANT POTENTIAL OF TINOSPORA CORDIFOLIA & MOMORDICA CHARANTIA

Rakhi Mishra[1*], Satkar Prasad[2]

Abstract

The free radicals called reactive oxygen or nitrogen species associated to the development of illnesses like cancer, stroke, and diabetes. Antioxidant from plants is a viable strategy for reducing the negative consequences of oxidative stress. Thus, this study deals with comparative phytochemical analysis & antioxidant potential of *Tinospora cordifolia* & *Momordica charantia.* The leaves of plant were collected & studied for organoleptic properties & then subjected to extraction by ethanol as solvent. Further Qualitative as well as quantitative studies were performed. The antioxidant potential of both plants was checked by DPPH & H_2O_2 assay. Results revealed that The Loss on Drying for *Tinospora cordifolia* & *Momordica charantia noticed as* 5.96% & 4.73% respectively. The Phytochemical analysis of *Tinospora cordifolia* revealed to have presence of alkaloid, glycoside, carbohydrate, tannin, flavonoid, steroid, protein & saponin. The *Momordica charantia* phytochemical test remarked almost similar results. In case of *Momordica charantia* the total phenol & flavonoid content was recorded as 4.33±0.055mg/100mg & 20.63±0.046 mg/100mg respectively. For the plant *Tinospora cordifolia* the phenol & flavonoid content estimated to have 14.33±0.055mg/100mg & 20.63±0.046 mg/100 mg respectively. The *Momordica charantia* seem to have IC 50 value of 80.32±0.100. While *Tinospora cordifolia* assessed to have IC 50 value of 80.25±0.055**.** So, it can be observed that both plants have almost similar antioxidant potential. The hydrogen peroxide radical scavenging assay IC 50 value for *Tinospora cordifolia & Momordica charantia* observed to have IC 50 as 3.48±0.050 & 3.51±0.206 respectively. Thus, from results it can stated as slightly higher antioxidant capability is present in *Momordica charantia*.

Keywords: Medicinal plants, Antioxidant, Phytochemicals, *Tinospora cordifolia, Momordica charantia*, DPPH, H_2O_2

[1*,2]Bhabha Pharmacy Research Institute, Bhabha University, Bhopal (M.P.)

***Corresponding Author:** Rakhi Mishra
*Bhabha Pharmacy Research Institute, Bhabha University, Bhopal (M.P.),
Email: mishrakrakhi0077@gmail.com

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Introduction

Fundamentally, free radicals are the root cause of a number of human diseases. They are produced as unevenness between prooxidant production and neutralisation, which leads to oxidative stress. Important free radicals called reactive oxygen or nitrogen species (ROS, RNS) hinder how humans function. ROS, including the hydroxyl radicals (OH-) and superoxide anion radicals (O2 -), are physiological metabolites. They are created by aerobic organisms as a by product of respiration, but their excessive levels have been associated to the development of illnesses like cancer, stroke, and diabetes (Riley,1994; Kunwar & Priyadarsini, 2011). They perform crucial physiological functions include regulating hormones, vascular tone, apoptosis, signal transduction, transcription factors, defence genes, and adaptive responses to enzymes. Much attention has recently been focused on the development of traditional medicines with potent antioxidant properties and minimal cytotoxicities (Jensen,2003; Poprac *et al*., 2017).

Antioxidants, when present at quantities lower than the substrate, dramatically slow down or stop the oxidation of oxidizable substrates. Antioxidants can be consumed as dietary antioxidants or produced in the body (e.g., reduced glutathione (GSH), superoxide dismutase (SOD), etc.) Exogenous (dietary, i.e., plant-based) antioxidants have long been found in plants. Almost all of the plant species in the world are thought to have medicinal use, and nearly all of them have excellent antioxidant potential. The discovery and subsequent isolation of ascorbic acid from plants first sparked interest in the exogenous plant antioxidants. Since then, increasing oxidative stress has drawn a lot of attention because it has been found to be a major contributing element in the onset and progression of many serious diseases, including cardiovascular and neurological disorders. A viable strategy for reducing the negative consequences of oxidative stress includes increasing the body's endogenous antioxidant defences or using exogenous antioxidant supplements (Reische *et al*., 2017; Halliwell,1996; Li *et al*., 2007; Kim *et al*., 2004).

The plant *Tinospora cordifolia* Hook, Guduchi/Amrita in local terms; Indian in English As a member of the Menispermaceae family, Tinospora is present in Bangladesh, Myanmar, Sri Lanka, and China. is a growing shrub with many twisted branches that spreads and mounts. *T. cordifolia* is employed in the ayurvedic medical system and possesses a wide range of therapeutic benefits these benefits include use in conditions such as rheumatism, anaemia, urinary disorders, skin illnesses, jaundice, diabetes, allergic conditions, and inflammation. Strong antiemetic properties make the root of *T. cordifolia* useful in treating intestinal blockage. A few researchers have also suggested that *T. cordifolia* can treat chronic fevers, boost energy and appetite, and relieve burning sensations (Spandana *et al*., 2013; Mittal *et al*., 2014; Mishra *et al*., 2013).

Another plant *Momordica charantia*, a climber from the Cucurbitaceae family, is also known as karela in Hindi and bitter gourd in English. *Momordica charantia* (MC) is widely used as a purgative, anthelmintic, contraceptive, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism, fever, and scabies in various systems of traditional medicine. Its use in treating diabetes and its consequences (nephropathy, cataracts, and insulin resistance), as an antibacterial and antiviral drug (including against HIV infection), as an anthelmintic, and as an abortifacient has been verified by more than 100 research conducted utilising cutting-edge methods. It has historically traditionally been used to treat peptic ulcers, but intriguingly, recent experimental investigations have shown that it may also be effective against *Helicobacter pylori*. The trials have most importantly demonstrated its effectiveness in treating a number of cancers, including lymphoid leukaemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumours, prostatic cancer, squamous cell carcinomas of the tongue and throat, human bladder carcinomas, and Hodgkin's disease. There are a few papers on the clinical application of MC in patients with cancer and diabetes that have demonstrated encouraging outcomes (Khalid *et al*., 2021; Daniel *et a*l., 2014; Gupta *et al*., 2011). Thus, keeping in view the medicinal uses of both the plants this study deals with comparative phytochemical analysis & antioxidant potential of both the plant species.

Materials & Methods

Plant material

The leaves of *Tinospora cordifolia* and *Momordica charantia* were collected from green vally nursery, Sambalpur (Odisha) in Feb, 2020.

Extraction by maceration process

Extraction of plant product of *Tinospora cordifolia* and *Momordica charantia* were done by

Maceration method. 100gm of dried plant material were exhaustively extracted with ethanolic solvent using maceration method.

Physicochemical parameters

The plant powder was evaluated for below mentioned physicochemical parameters (Rajurkar and Hande,2011).

Total ash value

A fine even layer of two grams of powder was put at the bottom of a pre-weighed platinum crucible. The crucible containing the powder was moved to a muffle furnace. The furnace's temperature was gradually raised to 450^{0}C. The powder was stored for 6 hours until the sample's ignition was complete. While the heating carried occurring, the crucible was weighed on an irregular basis. Once the sample was fully ignited, the crucible weight remained unchanged. After that, the crucible was withdrawn and allowed to cool to ambient temperature. The crucible's weight was then recorded, and the ash value was determined before it was removed from the furnace, cooled, and weighed. The proportion of total ash was computed.

Acid insoluble ash value

This value was determined using the ash obtained in the total ash value test. 25 ml of 2N hydrochloric acid was added to the ash, mixed and boiled for 5 minutes. The contents were filtered using ashless filter paper to collect acid insoluble ash. The material was subjected to ignition for 15 minute after washing in hot water. The weight of ash obtained was recorded and used to calculate acid insoluble ash.

Acid insoluble ash value

This value was calculated using the ash from the total ash value test. To the ash, 25 mL of 2N hydrochloric acid was added, stirred, and heated for 5 minutes. To capture acid insoluble ash, the contents were filtered using ashless filter paper. After washing in hot water, the material was ignited for 15 minutes. The ash weight was recorded and used to compute acid insoluble ash.

Water soluble ash value

The ash from the technique described in section 6.3.1 was used in this case. It was combined with 25 millilitres of water and boiled for 5 minutes. To capture water insoluble ash, the water was cooled and filtered through ashless filter paper. It was ignited again after being washed with hot water. The value was used to compute the ash content that was water soluble.

Water soluble extractive value

5 grams of the powder mixture were placed in a conical flask and macerated for six hours with 25 mL of distilled water. After then, the mixture was shaken for 18 hours. The filtrate was then put into a tared porcelain dish once it had been filtered. The filtrate-containing dish was then dried at 1050°C. The meal was then placed in a desiccator for around 30 minutes to cool. The weight of the dried filtrate was then measured with an electronic weighing scale and used to calculate the water soluble extractives.

Alcohol soluble extractive value

5 grams of the polyherbal mixture were placed in a conical flask and macerated for 6 hours in 25 ml ethanol (95%). The mixture was left at room temperature for 18 hours while being shaken frequently. The filtrate was put into a tared porcelain plate after being filtered. The filtrate-containing dish was then dried at 105°C. The meal was then placed in a desiccator for around 30 minutes to cool. The dried filtrate's weight was then recorded on an electronic weighing balance and used to calculate alcohol-soluble extractives.

Ether soluble extractive value

The procedure followed was same as that of section 6.3.5 except for the use of petroleum ether in place of water.

Total Phenolic content estimation:

Principal: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Singleton *et al*., 1999).

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 25- 125µg/ml was prepared in methanol.

Preparation of Extract:

1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoid content estimation:
Principal:
Determination of total flavonoids content was based on aluminium chloride method (Sahu and Saxena,2013).

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 125µg/ml were prepared in methanol.

Preparation of extract
1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

In-vitro antioxidant activity of ethanolic extract of *Tinospora cordifolia*, *Momordica charantia,* using DPPH method
DPPH scavenging activity was measured by the spectrophotometer (Mishra *et al*., 2012). Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Hydrogen Peroxide Scavenging Capacity
The ability of the *Tinospora cordifolia, Momordica charantia,* extracts to scavenge hydrogen peroxide was determined according to the method of Keser *et al* (2012). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

Results & Discussion
The result of organoleptic evaluation revealed that *Tinospora cordifolia* & *Momordica charantia* have cremish brown & brownish black colour respectively with bitter & pungent odour. The Loss on Drying for *Tinospora cordifolia* & *Momordica charantia noticed as* 5.96% & 4.73% respectively.

Different ash values were also estimated for *Tinospora cordifolia* & *Momordica charantia*. The maximum value of total ash was observed to be 10.54±0.58 % for *Momordica charantia*. Also, Acid insoluble, Ether soluble extractive value for *Momordica charantia* observed to be 1.84±0.54% & 1.42± 0.83% respectively.

In case of *Tinospora cordifolia,* Water soluble, Sulphated, Alcoholic, Aqueous extractive value detected to be 5.18 ± 0.36%, 0.93 ± 0.21%, 18.47±0.11%, 8.24±0.17%. Total Percentage yield *Tinospora cordifolia* & *Momordica charantia* witnessed as 12.23% & 13.45% respectively.

The Phytochemical analysis of *Tinospora cordifolia* revealed to have presence of alkaloid, glycoside, carbohydrate, tannin, flavonoid, steroid, protein & saponin. The *Momordica charantia* phytochemical test remarked positive results for Glycosides, Carbohydrates, flavonoid, steroid, protein & amino acid, resin & saponin.

From the result of total phenol & flavonoid content it was confirmed that *Tinospora cordifolia* contain more amount of theses constituents. In case of *Momordica charantia* the total phenol & flavonoid content was recorded as 4.33±0.055 mg/100mg & 20.63±0.046 mg/100mg respectively. For the plant *Tinospora cordifolia* the phenol & flavonoid content estimated to have 14.33±0.055mg/100mg & 20.63±0.046 mg/100 mg respectively.

The pharmacological activity specifically antioxidant activity was evaluated for both the plant with DPPH & by hydrogen peroxide radical scavenging methods. IC 50 value was separately calculated to know the antioxidant potential of the plant extract.

Here, ascorbic aid was used as standard which gave IC 50 value of 17.70±0.015. The result of DPPH assay revealed that *Momordica charantia* seem to have IC 50 value of 80.32±0.100. While *Tinospora cordifolia* assessed to have IC 50 value

of 80.25±0.055**.** So, it can be observed that both plant have almost similar antioxidant potential.

The hydrogen peroxide radical scavenging assay also employed ascorbic acid as standard. The IC 50 value for ascorbic acid was seen to be 5.46±0.270 in this case. Further The IC 50 for *Tinospora cordifolia & Momordica charantia* observed to have IC 50 as 3.48±0.050 & 3.51±0.206 respectively. Thus from results it can stated as slightly higher anioxidant capability is present in *Momordica charantia*.

Table 1: Results of Organoleptic Evaluation

Test	***Tinospora cordifolia***	***Momordica charantia***
Colour	Creamish brown	Brownish black
Odour	Characteristic	Characteristic
Taste	Bitter	Pungent

Table 2: Results of loss on drying (LOD)

Test	***Tinospora cordifolia***	***Momordica charantia***
Loss on Drying (LOD) %w/w	5.96	4.73

Table 3: Ash value of the selected plants

Test	***Tinospora cordifolia***	***Momordica charantia***
Total ash(%w/w)	7.84 ± 0.72	10.54±0.58
Water soluble(%w/w)	5.18 ± 0.36	4.94±018
Acid insoluble (%w/w)	0.56 ± 0.13	1.84±0.54
Sulphated (%w/w)	0.93 ± 0.21	0.82±0.46
Alcoholic (%w/w)	18.47±0.11	13.41±0.24
Aqueous (%w/w)	8.24±0.17	5.15±0.08
Ether soluble extractive value(%w/w)	0.93± 0.26	1.42± 0.83

Table No. 4: Results of Percentage yield of Different Extracts

S. No	**Methanolic Extract**	**Percentage yield**
1.	***Tinospora cordifolia***	12.23%
2.	***Momordica charantia***	13.45%

Table 5: Phytochemical analysis of *Tinospora cordifolia* (Gilloy)

S.NO.	Identification Test	Test name	Present
1	Alkaloids	Mayer's test	-
		Dragendorff's test	+
		Wagner's test	+
2	Glycosides	Killer-killani test	
		Baljet test	+
		Foam test	+
3	Carbohydrates	Molisch's test	+
		Fehling test	+
		Benedict test	+
4	Tannins	Vanillin-HCL test	+
		Gelatin test	+
		Ferric chloride test	-
5	Flavonoids	Lead acetated test	+
		Shinoda test	-
		Alkaline reagent test	+
6	Steroids	Libermamm-Burchard test	-
		Salkowski test	+
7	Protein and amino acid	Biuret test	+
		Precipitation test	+
		Xanthoproteic test	-
9	Saponins	Froth test	-
		Foam test	+

(+) = Present, (–) = Absent

Table 6. Phytochemical analysis of *Momordica charantia* (karela)

S.NO.	Identification Test	Test name	Results
1	Alkaloids	Mayer's test	-
		Dragendorff's test	-
		Wagner's test	-
2	Glycosides	Killer-killani test	
		Baljet test	+
		Foam test	+
3	Carbohydrates	Molisch's test	
		Fehling test	+
		Benedict test	+
4	Tannins	Vanillin-HCL test	-
		Ferric chloride test	-
5	Flavonoids	Lead acetated test	-
		Shinoda test	+
		Alkaline reagent test	-
6	Steroids	Libermamm-Burchard test	+
		Salkowski test	+
7	Protein and amino acid	Biuret test	+
		Precipitation test	+
		Xanthoproteic test	-
8	Resins	Colour detection with ferric chloride	+
		Turbidity test	+
9	Saponins	Froth test	+
		Foam test	+

(+) = Present, (–) = Absent

Table No. 7: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (Mean) λ max=760 nm
1	25	0.660
2	50	0.765
3	75	0.854
4	100	0.956
5	125	1.051

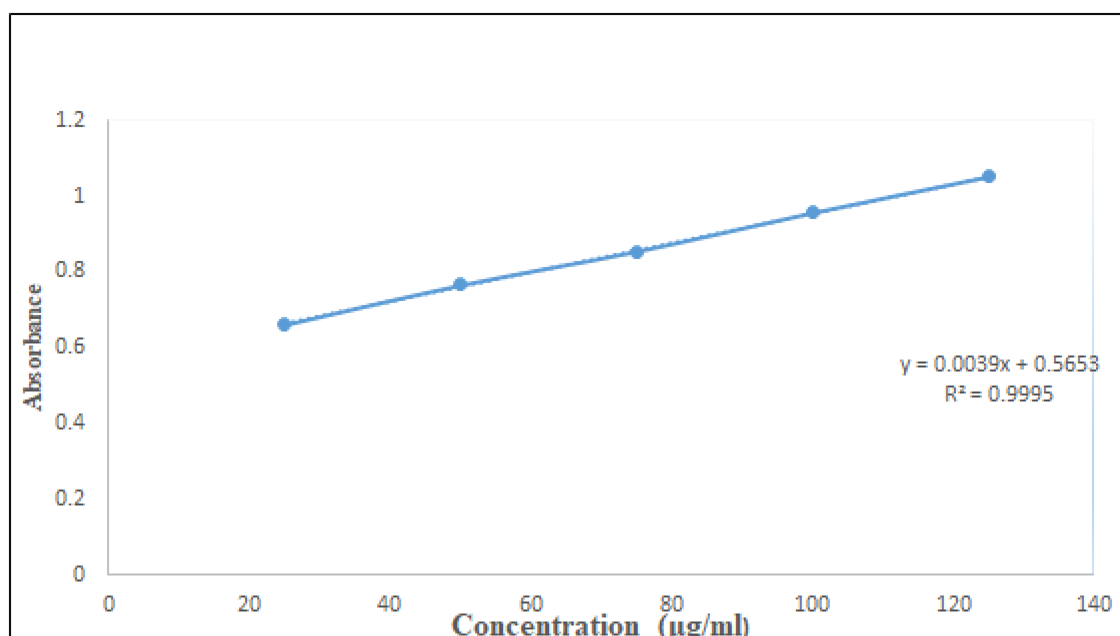

Fig. No. 7.1: Calibration curve of Gallic acid

Table No. 8: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance (Mean) λmax=420 nm
1	25	0.234
2	50	0.448
3	75	0.658
4	100	0.869
5	125	1.102

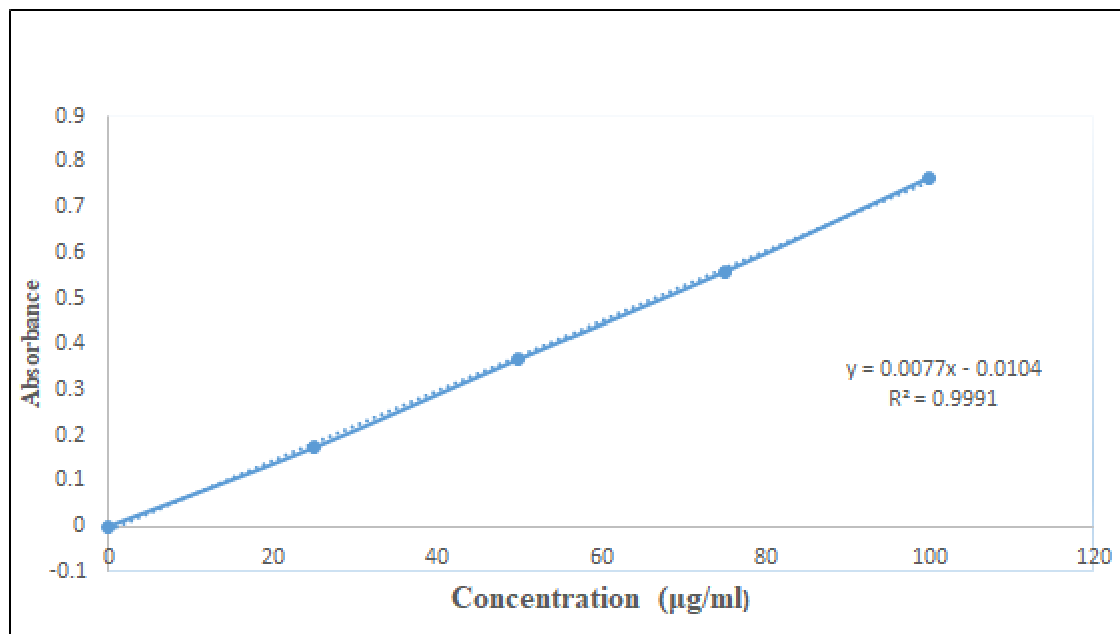

Fig. No. 7.2: Calibration curve of Quercetin

Table No. 9: Total Phenolic and flavonoids content in different Plant extract

S. No.	Different plant Extracts	Total Phenol (mg/gm)	Total Flavonoids (mg/gm)
1.	*Tinospora cordifolia*	24.74 ± 0.26	25.60±0.10
2.	*Momordica charantia*	14.33±0.055	20.63±0.046

Table 10: Effect of selected plant extracts and ascorbic acid on DPPH radical scavenging activity

Concentrations (µg)	Ascorbic Acid	*Tinospora cordifolia*	*Momordica charantia*
10	42.33±0.733	13.20±0.075	18.26±0.445
20	48.18±0.473	21.29±0.577	20.39±0.005
40	65.34±0.449	33.10±0.330	33.93±0.596
60	69.85±0.231	37.33±0.449	47.86±0.330
80	80.26±0.896	44.99±0.385	55.17±0.627
100	87.294±0.858	55.50±0.295	66.57±0.861
IC50	17.70±0.015	80.25±0.055	80.32±0.100

Table 11: Effect of selected plant extracts and ascorbic acid on hydrogen peroxide radical scavenging activity

Concentrations (µg)	Ascorbic Acid	*Tinospora cordifolia*	*Momordica charantia*
10	15.37±0.561	25.97±0.634	28.25±0.678
20	32.11±0.548	45.70±0.580	38.92±0.580
40	54.16±0.501	53.98±0.426	54.83±0.722
60	59.23±0.457	68.74±0.476	67.86±0.927
80	68.36±0.735	77.14±0.274	75.71±0.708
100	83.25±0.505	91.25±0.395	86.97±0.318
IC50	5.46±0.270	3.48±0.050	3.51±0.206

Conclusion

In conclusion, the current study compared the phytochemical and antioxidant capabilities of the ethanolic-extracted plants *Tinospora cordifolia* and *Momordica charantia*. Numerous bioactive components in both plants are present, according to phytochemical tests. The overall phenol and flavonoid content, which is responsible for the majority of the pharmacological effects, was also very high. The derived plant extracts demonstrated dose-dependent scavenging efficacy against DPPH and H2O2 radicals, with the H2O2 assay exhibiting the greatest inhibitory extent. This study adds to the expanding body of research suggesting that the phenolic compound-rich plants *Tinospora cordifolia* and *Momordica charantia* may have positive effects on human health. The exact health protective effects of each constituent may be determined by further research that isolates and tests each constituent in the extracts, as well as through various combination studies. This information may then be used to guide the development of specialist *Tinospora cordifolia* and *Momordica charantia* extracts for health promotion.

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