JNCS
Journal of Nepal Chemical Society
March/April 2023, Vol. 43, No. 2, 91-102
ISSN: 2091-0304 (print)
DOI: https://doi.org/10.3126/jncs.v43i2.53348
RESEARCH ARTICLE

Comparative Phytochemical and Biological Study of *Tinospora cordifolia* (Thunb.) Miers and *Justicia adhatoda* L.Plants Collected from West Rukum of Nepal

Biraj Oli[1], Gyanendra Ghimire[1], Bijay Dhakal[1], Neetu Amatya[1], Deepjyoti Adhikari[2], Netra Lal Bhandari[1]*

[1]Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

[2]Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal

*Corresponding E-mail: netra.tu.edu@gmail.com

Submitted: 12 Dec 2022, Revised : 7 Feb 2023, Accepted: 12 Feb 2023

Abstract

Medicinal plants contained an impressive number of modern drugs and are believed to be a precious natural reservoir that has been continuously studied for their pharmacological activities against various ailments. *Tinospora cordifolia* and *Justicia adhatoda* are widely used shrubs in folk and ayurvedic systems of medicine. Phytochemical screening of different fractions of the extract of the bark of *T. cordifolia* and leaf, stem, and flowers of *J. adhatoda* was done and results showed the presence of alkaloids, flavonoids, phenolics, glycosides, terpenoids in both plants. The antibacterial potency of medicinal plant extracts has been tested against *Bacillus subtilis* ATC6051 and *Enterococcus faecalis* ATCC29212 by disc diffusion assay. The leaves extract of *J. adhatoda* showed good antibacterial activities towards both the *Enterococcus faecalis* and *Bacillus subtilis* bacteria, however, the extract of *T. cordifolia* was found not soo effective with those bacteria. The methanol extract of *T. cordifolia* stem showed the strongest 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity with IC_{50} values 8.213µg/mL comperatively closer to standard ascorbic acid (22.451 µg/mL). Furthermore, results showed that the *T. cordifolia* is a good source of antioxidant as compared to the *J. adhatoda*. The total phenolic content (TPC) was the highest in methanolic extract of *T. cordifolia* (46.463 mg GAE/g extract) while the *J. adhatoda* had lower values (31.167 mg GAE/g extract) by taking gallic acid as a standard. The total flavonoid content (TFC) was the highest in methanol extract of *J. adhatoda* leaves (13.030 mg QE/g extract) while *T. cardifolia* had lower values (2.112 mg QE/g extract) which were determined by taking quercetin as a standard. The result revealed that the TPC is higher in *T. cordifolia* and TFC value higher in *J. adhatoda* and that can be correlated with the antimicrobial and antioxidant properties of phytoconstituents although the plants have been used for the similar ethnomedical purpose in society.

Keywords: *Phytochemical screening, Tinospora cordifolia, Justicia adhatoda compounds, antibacterial activity, IC_{50} value*

Introduction

Since the prehistoric era, nature is considered as a pivotal source of novel compounds and medicinal agents in the field of drug discovery. Even in the present time, an impressive number of modern drugs are isolated from natural sources [1]. According to WHO, 80% of the world's population relies prominently on traditional medicine involving the use of plant extract or their active constituents [2], whilst around 35,000-70,000 plants species are used for medicinal purposes globally and approximately 6,500 species only in Asia [3, 4]. In Nepal, at least 1,600 to 1,900 species of plants are generally used in traditional medicinal practice [5]. Up to 50% of

approved drugs and more than 60 % of anticancer and anti-infective drugs during the last 30 years were derived purely from natural products or were inspired by molecules derived from natural sources, including semi-synthetic analog and they play an important role in drug development programs of the pharmaceutical industry [6].

Nepal with its mega-biodiversity and knowledge of rich ancient traditional systems of medicine provides a strong base for the utilization of a large number of plants in general health care and alleviation of common ailments of the people [7]. Among such indigenous medicinal plants, *Tinospora cordifolia* (Nepalese local name: Gurjo*)* and *Justicia adhatoda* (Nepalese local name: Ashuro) plants are important medicinal plants used for the treatment of various diseases. The various characteristics of plants such as antimicrobial properties, the emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin [8]. These plants make many chemical compounds to protect themselves against fungi, and bacteria, this act in the same way on the human body as allopathic drugs [9]. Furthermore, it has been reported that plants possessing free radical scavenging activity are known to have organ-protective effects [10].

T. cordifolia (Family: Menispermaceae) is a natural, extensively spreading glaborous, perennial deciduous twine with succulent stems and papery bark and grows at temperature of 25 to 45^{o} C. It is native to the tropical region of India [11-13] and is profoundly used as a rejuvenator, anti- aging, antispasmodic, antipyretic, anti-inflammatory, anti-complementary and immunomodulatory, and antioxidant properties [14]. The therapeutic treatment of several diseases like cardiac diseases, leprosy, helminthiasis, skin diseases, chronic diarrhea, diabetes, jaundice, urinary problems rheumatoid arthritis, mitigate the negative effects of chemotherapy. It acts by destroying and immune-boosting properties via its Phytoactive chemical extracts [15] such as alkaloids, diterpenoids, sesquiterpenoids, lactones, glycosides, steroids, phenolic, aliphatic compounds [16]. Similarly, *J. adhatoda* (Family: Acanthaceae) is an ayurvedic shrub, widespread through the tropical regions of southeast Asia, especially in the lower Himalayan regions [17, 18]. Typically described as erect or scandent, perennial, evergreen and highly branched shrub (1.0m to 2.5m height), its flowers, leaves, and root extracts have expectorant and antispasmodic properties [8]. Its leaves contain alkaloids, flavonoids, glycosides, cardiac glycosides, coumarins, hydroxyanthraquinones, tannins, phlorotannins, proteins, xanthoproteins, steroids, and phenols [19]. It is extensively used to treat a wide variety of chronic as well as infectious respiratory diseases like colds, coughs, asthma, bronchitis, and tuberculosis [20].

Owing to the fact that, Nepal is a king-sized country having biodiverse medicinal herbs, albeit merely limited research has been carried out to exhibit its significance, antimicrobial and antioxidant property scientifically. There is no scientific system for collecting and regenerating these plants, several such high-value plants have either been completely lost or have become endangered [21]. This study aims to carry out the comparative phytochemical study of *T. cordifolia* and *J. adhatoda* that can be correlated with antimicrobial and antioxidant activities owing to their similar ethnomedicinal values to justify with scientific evidence.

Materials and Methods

Sample Collection

The herbarium samples of *T. cordifolia* and *J. adhatoda* was collected from West-Rukum district of Nepal between December to February. Plants were taken for authentication to the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu via comparison with herbarium species present there.

Collection of Plant Extract

Initially, the bark of *T. cordifolia* and the stems, leaves, flowers of *J. adhatoda* were washed meticulously with distilled water and air dried for 7 days, then kept in an incubator at 37°C for 3-4 days, and ground into fine powder. Finally, about 200 g of *T. cordifolia,* and

150 g of *J. adhatoda* were taken in separate soxhlet extractors, and extracts were collected separately in a in a flask containing 500 mL hexane, chloroform, ethyl acetate, and methanol solvents respectively. The solvent was removed from the extract, and concentrated under reduced pressure in a rota vapour to get viscous liquid as phytochemical extracts.

Phytochemical Analysis of Plant Extract

Phytochemical screening of the selected plant materials involved the selective and successive extraction was primarily employed following the standard procedure reported by Dhote (2015) [22]. The presence of main groups of natural constituents in the different extracts was analyzed by using different specific reagents [23]. Also, thin layer chromatography (TLC) was employed for hexane, ethyl acetate, and chloroform extracts in solvents mixture of hexane and ethyl acetate at three different ratios (5: 5, 2.5: 7.5, 7.5: 2.5). The spots were visualized in daylight as well as under UV light, both at 254 nm and 366 nm. The chromatogram was further visualized by spraying with 1% ferric chloride solution in methanol.

Determination of Antimicrobial Activity of Plant Extract

Antibacterial screening of the plant extracts was performed by Agar-well diffusion method in Muller Hinton agar (MHA) via lawn culture method and minimal bacterial concentration of the extracts was determined by the micro-dilution method using plant fraction serially diluted in sterile Nutrient Broth (NB) as per Clinical and Laboratory Standards Institute (CLSI) guideline [24]. The effectiveness of antimicrobial substance was evaluated by determination of zone of inhibition (ZOI) as given by Cavalieri S. J. The control microbial strains of *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC6051) were obtained from Polytechnic Research Institute of Nepal (PORIN), Kathmandu, Nepal. The standard inoculum of test organisms was made by dissolving in dimethyl sulphoxide (DMSO). The inoculum of bacteria was incubated overnight in MHA plate. Wells were made via sterile cork border and 15 µL of the working solution of the plant extracts was loaded into the respective wells with the help of a micropipette. Ofloxacin was used as a control in the separate well and plates were incubated overnight at 37°C. ZOI produced by the antibacterial activity of plant extract was measured by the use of a scale.

Determination of Antioxidant Activity

DPPH radical scavenging activity was accessed for the determination of the antioxidant activity of plant extracts with ascorbic acid as standard. About 0.2 mM solution of DPPH in 100% methanol was prepared by dissolving 78 mg of DPPH 1000 mL methanol and kept overnight at 4°C. The different concentrations of 20, 40, 60, 80, and 100 µg/mL of each extract i.e acetone and methanol of *T. cordifolia* and *J. adhatoda* were prepared by serial dilution of the stock solution of respective extracts. To each 2 mL concentration of the extracts, 2 mL of 0.2 mM methanolic DPPH solution was added. A control was prepared by mixing 2 mL of distilled water and 2 mL of 0.2 mM methanolic DPPH solution. The absorbance was measured at 517 nm using a spectrophotometer against the blank solution. The radical scavenging activity with calibration was prepared and expressed as percentage radical scavenging using the following equation 1.

$$\%\ radical\ scavanging\ ativities = \frac{\mathrm{Abs_{control}} - \mathrm{Abs_{conc}}}{Abs_{\mathrm{control}}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots 1$$

Where, Abs control = absorbance of control

Abs conc = absorbance of the solution of each concentration

The IC50 value is the concentration of sample required to scavenge 50% of DPPH free radical and the crude extract was compared with that of ascorbic acid. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Determination of Total Phenolic Content (TPC)

The TPC of all selected plant extracts was estimated using Folin Ciocalteu reagent involving gallic acid standard based on oxidation-reduction reaction. The procedure carried out for the total phenol content was based on the standard procedure reported by Waterhouse (2009) [25]. TPC content compound concentration in extract was expressed as milligrams

of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract which was calculated in all the extracts separately using the following formula 2.

$$C = \frac{cV}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 2$$

Where, C = Total phenolic content compounds in mg/g, in gallic acid equivalent (GAE) c = Concentration of gallic acid established from the calibration curve in mg/mL V = Volume of extract in mL, m = Weight of plant extract

Determination of Total Flavonoid Content (TFC)

TFC of the selected plant extracts was determined according to the aluminum chloride colorimetric method involving quercetin as standard as described by Kalita, *et.al*, (2013) [26]. The total flavonoid content was calculated in all the extracts separately using formula 3.

$$C = \frac{cV}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 3$$

Where, *C* = total content of flavonoid compounds in mg/g, in Quercetin equivalent (QE),

c = concentration of Quercetin established from the calibration curve in mg/mL,

V = the volume of extract in mL, *m* = the weight of plant extracts.

Results and Discussion

Phytochemical Screening

The result obtained from the phytochemical screening of *T. cordifolia* and *J. adhatoda* in different solvents is shown in Table 1.

In the present study, it was found that the stem of *T. cordifolia* contained alkaloids, glycosides, steroids, proteins,andcarbohydratesasactivephytoconstituents. The phytochemical study of *J. adhatoda* revealed the presence of alkaloids, flavonoids, polyphenols, terpenoids, and carbohydrates as phytochemical constituents as in Table 1. The results of quantitative estimation showed that the plant extracts are rich sources of secondary metabolites.

Thin Layer Chromatography

The thin-layer chromatographic technique is a useful analytical tool for the isolation and identification of organic compounds. The TLC for alkaloids showed better separation in the ethyl acetate extract of both *T. cordifolia and J. adhatoda* were examined by TLC in solvents mixture (ethyl acetate: hexane) at different ratios and the spots were visualized in daylight, under UV chamber, both at 254 nm and 366 nm. The TLC profile of the ethyl acetate extract of both plants has been observed clearly and visualized in

Table 1: *Qualitative chemical analysis of T. cordifolia and J. adhatoda extract*

Group of Compounds	Name of Tests	*T. cordifolia*				*J. adhadota*			
		Hexane extract	EtOAc extract	$CHCl_3$ extract	MeOH extract	Hexane extract	EtOAc extract	$CHCl_3$ extract	MeOH extract
Basic Alkaloids	i. Dragendroff	++	++	++	+	+	+	+	+
	ii. Mayers	-	+	++	+	+	+	+	+
	iii. Wagner	++	+	+	+	-	-	-	+
Flavonoids	i. Sinoda	+	+	+	+	++	++	++	++
	ii. Sibata	+	+	+	+	+	+	+	+
Coumarins		+	+	+	+	+	+	+	+
Saponins	Foam test	+	-	-	+	+	+	+	+
Polyphenols	$FeCl_3$ test	+	+	+	+	-	+	+	+
Terpenoids	Salkowski test	-	-	+	-	-	-	+	+
Cardiac Glycosides	Killer killani	+	+	+	+	-	-	-	-
Proteins	Xanthoproteic	+	+	+	+	-	-	-	+
Carbohydrates	Molish test	+	+	+	+	+	+	+	+

(+); presence of phytochemicals, (-); absence ofphytochemicals

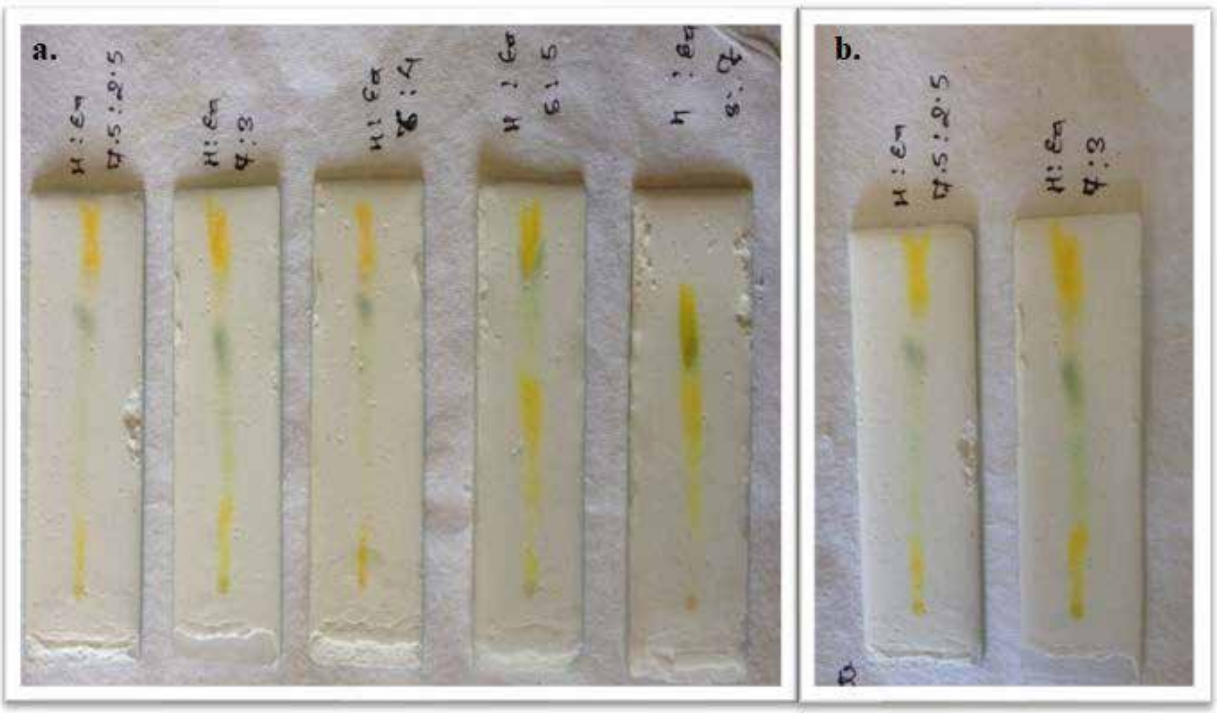

***Figure 1**: TLC plates of the solvent system were dipped in natural product (NP) reagent [Solvent system for TLC for T. cordifolia extract; ethyl acetate: hexane ratios 2.5:7.5, 3:7) and solvent system for J. adhatoda extract; ethyl acetate: hexane (2.5:7.5, 3:7, 4:6, 5:5, 7.5:2.5)].*

the photograph of the TLC chromatogram under the different solvent systems as in Figure 1.

The chromatogram was further visualized by spraying with 1% ferric chloride solution in methanol. The solvent systems used for the development of the chromatogram, Rf values, and band spot are given in Table 2.

Antimicrobial Activity

The antimicrobial activity of *T. cordifolia* and *J. adhatoda* against *Enterococcus faecalis* and *Bacillus subtilis* could be attributed to the secondary metabolites and the phytochemicals present in them such as polyphenols, alkaloids, flavonoids, tannins, coumarins, terpenoids, lectins and polypeptides [27]. The result of the antibacterial activity of extracts in hexane, acetone, chloroform, and methanol has been observed and reported. Acetone extract of *J. adhatoda* leaves shows good ZOI on both bacteria with 14mm and 12mm on *Enterococcus faecalis* and *Bacillus subtilis* comparable with hexane extract (i.e, 14mm and 10mm respectively) as in Figure 2.

***Table 2:** Calculations of retention factor values of T. cordifolia and J. adhatoda in ethyl acetate extract*

Plants name	Extract	The solvent distance (cm)	The solute distance (cm)	R_f Value	Color of spot
T. cordifolia	Ethyl acetate	4.9	2.6	0.53	Light green
			3.5	0.71	Dark green
			4.7	0.96	Yellow
J. adhatoda	Ethyl acetate	4.8	2.5	0.52	Light yellow
			3.2	0.67	Light green
			4.3	0.89	Dark green
			4.5	0.94	Yellow

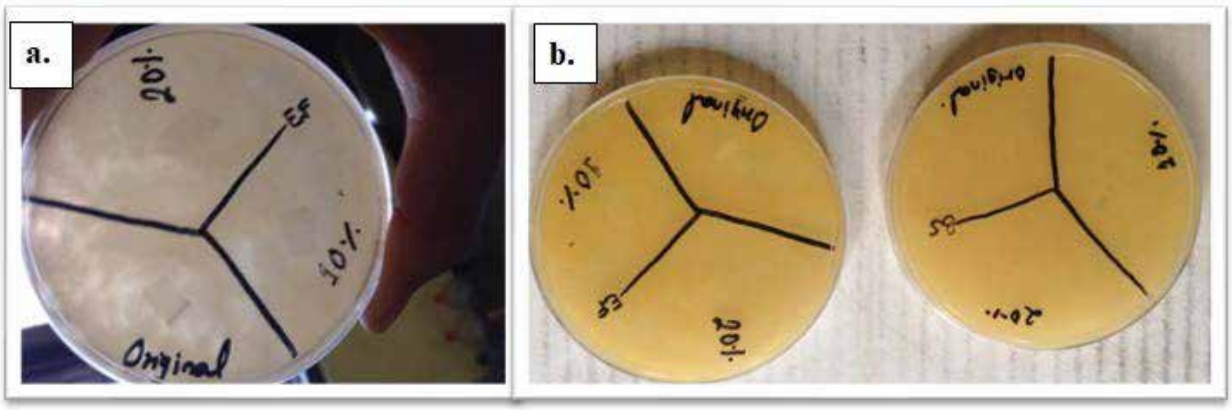

Figure 2: *Antimicrobial activity analysis with different ZOI of plant extract of T. cordifolia (a) and J. adhatoda (b)*

However, only the hexane extract of *T. cordifolia* showed active antimicrobial activity (10mm zone of inhibition only with *Bacillus subtilis* bacteria at 200 mg/mL, also similar to hexane extract of *J. adhatoda* (leaves extract) but the methanolic, chloroform and acetone extract of *T. cordifolia* towards both bacteria did not show any response. The antimicrobial activity of different extracts with various bacteria has been summarized in Table 3 and Figure 2.

Antioxidant Activity

DPPH Radical Scavenging Activity

DPPH has been widely used to evaluate the free radical scavenging effect of various antioxidant substances. In the DDPH assay, the antioxidants were able to reduce the stable radical DPPH to yellow-colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. With this method, it was possible to determine the antiradical power of an antioxidant by measuring a decrease in the absorbance of DPPH at 517nm. Resulting in a color from purple to yellow, the absorbance decreased when the DPPH was scavenged by antioxidants through the donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule had an absorbance at 517nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

Table 3: *Antimicrobial activity with different ZOI of T. cordifolia and J. adhatoda plants extract in different solvents at 200 mg/mL*

Plant extracts	Bacteria	ZOI (mm) of *T. cordifolia*	ZOI (mm) of *J. adhadota*		
		Stem extracts	Stem extract	Leaves extract	Flower extract
Hexane	*E. faecalis*	0	0	0	0
	B. subtilis	**12**	**14**	**10**	**10**
Acetone	*E. faecalis*	0	0	**14**	0
	B. subtilis	0	0	**12**	0
Chloroform	*E. faecalis*	0	0	0	0
	B. subtilis	0	0	0	0
Methanol	*E. faecalis*	0	0	0	0
	B. subtilis	0	0	0	0

Table 4: *Percentage of radical scavenging with different concentrations*

Plants extract	% Radical scavenging activity					
	0	**20**	**40**	**60**	**80**	**100**
T. cordifolia	0	19.213	24.312	29.72	36.038	45.102
J. adhatoda	0	8.023	14.135	19.286	24.825	28.512
Ascorbic acid	0	72.573	80.615	87.507	93.538	98.277

The comparison of percentage radical scavenging between different plant extracts and as standard is shown in Table 4.

The comparison of percentage radical scavenging at a different concentration between plant extract and ascorbic acid as standard has been shown in Figure 3.

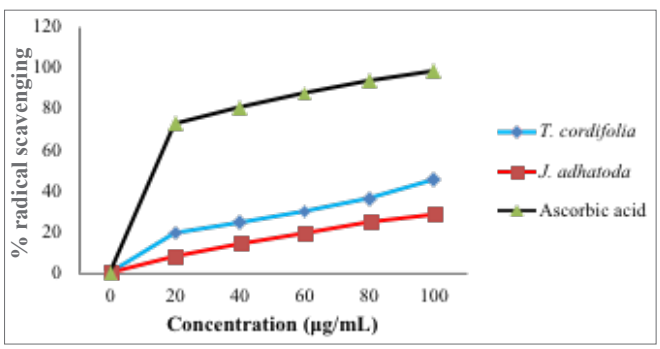

Figure 3: *A plot of percentage radical scavenging activity vs concentration of methanolic extract of T. cordifolia stem, J. adhatoda leaves, and ascorbic acid*

The linear regression of the percentage of radical scavenging versus concentration was used for the calculation of the concentration of each plant extract required for 50% inhibition of DPPH activity (IC_{50}). The antioxidant potential is in an inverse relation with IC_{50} value, lower value of IC_{50} indicates high antioxidant potential. The IC_{50} values of the plant extracts along with the standard ascorbic acid are tabulated in Table 5 and also in Figure 4.

Table 5: *Comparision of DPPH antioxidant and IC_{50} values of different plant extracts with standard ascorbic acid*

Sample (Methanolic Extract)	IC_{50} Values (µg/mL)
Tinospora cordifolia	8.213
Justica adhatoda	102.431
Ascorbic acid	22.451

Among the selected plants, methanolic extracts leaves of *T. cordifolia* showed higher IC_{50} value as compared to other extracts. More conveniently, the above table is represented in the bar graph in Figure 4.

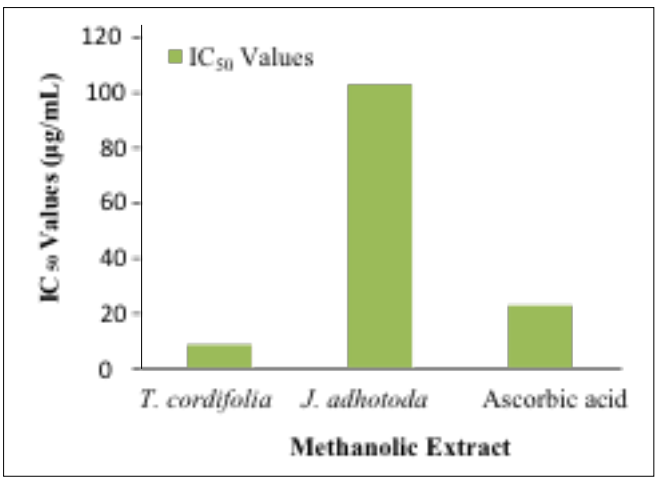

Figure 4: *Free radical scavenging activity (IC_{50}) in different plant extracts*

Table 5 and Figure 4 showed that the plant extracts have antioxidant potential as their IC_{50} values were found to be close to standard ascorbic acid. The methanol extract of steam showed the strongest DPPH radical scavenging activity with IC_{50} values 8.213 µg/mL of *T. cordifolia* which is close to standard ascorbic acid. The methanolic extract of leaves of *J. adhatoda* showed fewer antioxidant properties as compared to methanolic extracts of *T. cordifolia*. Results showed that the phenolic compounds known to possess high antioxidant activity are commonly found in fruits, vegetables, and herbs. Many studies revealed that the antioxidant activity of phenolic compounds is due to their redox properties, which allow them to act as reducing agents, singlet oxygen quencher, hydrogen donators, and chelating agents of metal ions. A significant relationship between antioxidant potential and total phenolic content was observed indicating that phenolic compounds might be the major contributor to the antioxidant potential [28].

Estimation of Total Phenolic Content (TPC)

The total soluble phenols present in various extracts of *T. cordifolia* and *J. adhatoda* were determined by using the Folin-Ciocalteu reagent (FCR) colorimetric method based on oxidation- reduction reaction.

The absorbance values of each extract at different concentrations (20 µg/mL, 40µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL) were recorded at 760 nm by using a spectrophotometer. The TPC in the plant extracts taken under study was calculated by using regression equation y = 0.012x+0.0085, R^2 = 0.9966, of the curve obtained from the above graph followed by the formula C= cV/m and expressed as mg GAE per g of extract in dry weight. The TPC of different plant extracts (mg gallic acid equivalent per g dry extract) are tabulated in Table 6 and Table 7.

The results demonstrated that the total phenolic content was highest in the methanolic extract of *T. cordifolia* (46.463 mg GAE/g extract) while the *J. adhatoda* had lower values (31.167mg GAE/g extract) as presented in Figure 6. Although a quantitative determination of phenolic compounds in plant extracts is hampered by their structural complexity, diversity, nature of analytical assay method, selection of standard, and presence of interfering substances.

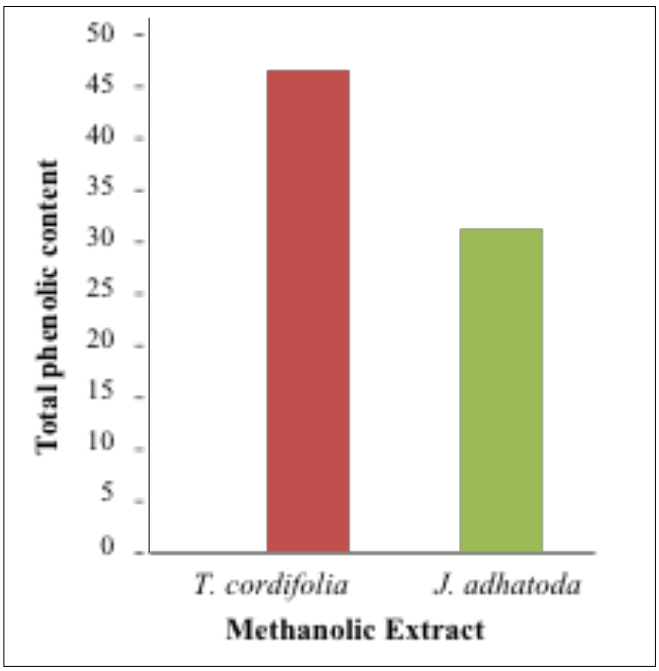

Figure 5: *Comparative study of TPC of T. cordofolia and J. adatoda*

The plant containing high phenolics can be used as foodstuffs, preservatives, etc. directly or indirectly by the human community. They have also been implicated as anticarcinogenic, antimicrobial, antimutagenic, antiangiogenic, and anti-inflammatory agents besides

Table 6: *Total phenolic content of methanol extract of T. cordifolia*

Sample solution (µg/mL)	Wt. of dry extract per mL m(g)	Absorbance	GAE conc. C (µg/mL)	GAE conc. C (mg/mL)	TPC as GAE (= cxV/m)	Mean
20	0.001	0.020	0.000020	0.020	47.712	
40	0.001	0.030	0.000030	0.030	45.325	
60	0.001	0.042	0.000042	0.042	46.031	**46.463**
80	0.001	0.053	0.000053	0.053	47.035	
100	0.001	0.064	0.000064	0.064	46.213	

Table 7: *Total phenolic content of methanol extract of leaves of J. adhatoda*

Sample solution (µg/mL)	Wt. of dry extract per mL m(g)	Absorbance	GAE conc. C (µg/mL)	GAE conc. C (mg/ml)	TPC as GAE (=cxV/m)	Mean
20	0.001	0.016	0.00002	0.016	32.251	
40	0.001	0.022	0.00002	0.022	29.921	
60	0.001	0.031	0.00003	0.031	31.546	**31.167**
80	0.001	0.039	0.00004	0.039	31.867	
100	0.001	0.044	0.00004	0.044	30.251	

their use in treating critical diseases like depression, cancer, microbial infections, lipid-related diseases, etc [28].

Estimation of Total Flavonoid Content

The total flavonoid contents in the extracts were estimated by using an aluminum chloride colorimetric assay. The total flavonoids present in different extracts of different plants were estimated by a standard procedure using the quercetin standard.

The graph shows the linear relationship between absorbance and concentration. The absorbance values of each extract taken under study at different concentrations (0.20 mg/mL, 0.40 mg/mL, 0.60 mg/mL, 0.80 mg/mL, and 1.0 mg/mL) were recorded at 510 nm by using a spectrophotometer. The TFC in the plant extracts taken under study was calculated by using regression equation y = 0.013x+0.0193, R^2 = 0.9986, of the curve obtained from the above graph followed by the formula cV/m and expressed as mg QE per g of extract in dry weight. The TFC of different plant extracts (mg quercetin equivalent per g dry extract) are tabulated in Table 8 and Table 9.

More conveniently Figure 6 represents the comparative study of the total flavonoid content was highest in the methanol extract of *J. adhatoda* leaves (13.030 mg QE/g extract) while *T. cardifolia* had lower values (2.112 mg QE/g extract).

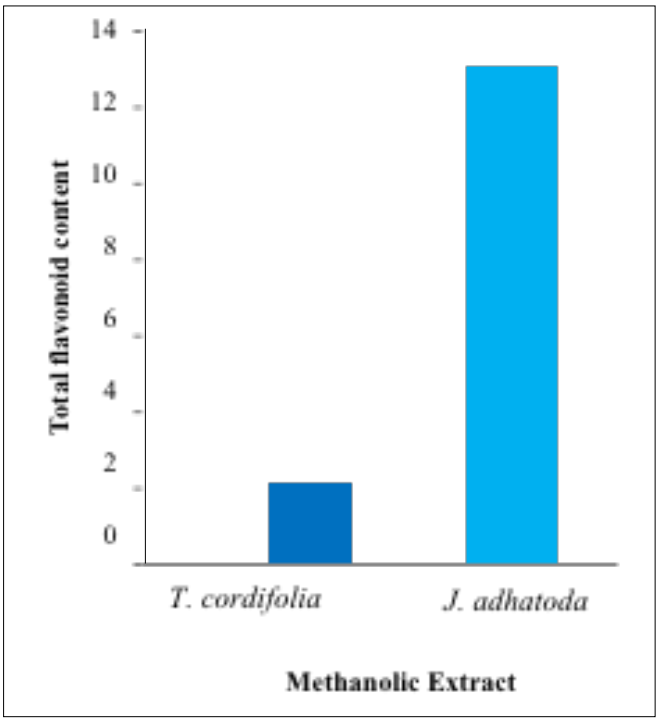

Figure 6: *Total flavonoid content in different methanolic plant extracts*

Flavonoid compounds have been known to possess high antioxidant properties due to their free radical scavenging properties. It has been reported that

Table 8: *Total flavonoid content of methanolic extract of Tinospora cordifolia*

Sample solution (µg/mL)	Wt. of dry extract per mL m(g)	Absorbance	QE conc. C (µg/mL)	QE conc. C (mg/mL)	TFC as QE (=cxV/m)	Mean
20	0.001	0.019	0.00002	0.019	2.134	
40	0.001	0.020	0.00002	0.020	1.915	
60	0.001	0.021	0.00002	0.021	2.013	**2.112**
80	0.001	0.022	0.00002	0.022	2.513	
100	0.001	0.022	0.00002	0.022	1.985	

Table 9: *Total Flavonoid Content of methanol extract of Leaves of Justicia adhatoda*

Sample solution (µg/mL)	Wt. of dry extract per mL m(g)	Absorbance	QE conc. C (µg/mL)	QE conc. C (mg/mL)	TFC as QE (=cxV/m)	Mean
20	0.001	0.022	13.07	0.013	13.07	
40	0.001	0.026	13.076	0.013	13.076	
60	0.001	0.029	12.949	0.012	12.949	**13.0304**
80	0.001	0.033	13.365	0.013	13.365	
100	0.001	0.035	12.692	0.012	12.692	

extract containing a large amount of polyphenol content possesses a greater antioxidant activity. More conveniently, total flavonoid content (TFC), total phenolic content (TPC), and DPPH radical scavenging activity (IC_{50} value) in different plant extracts are represented as a summary in Figure 7.

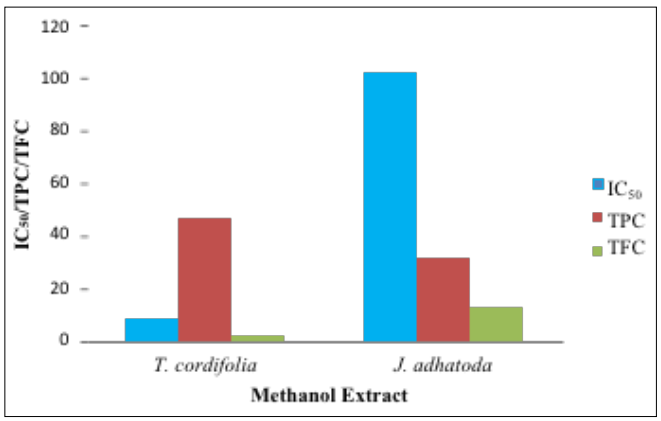

Figure 7: *TFC, TPC, and IC_{50} values in different methanolic plant extracts*

The bar graph depicts that TPC is higher in *T. cordifolia* so it would have better antioxidants, on the other hand, TFC and IC_{50} values are higher in leaves of *J. adhatoda* so it could have less scavenging ability but it reflects more antimicrobial activity than *T. cordifolia*.

Conclusion

The scientific research on *T. cordifolia* and *J. adhatoda* suggests a huge biological potential for these plants. The *T. cordifolia* stem and *J. adhatoda* leaves, flower, and stem revealed the presence of alkaloids, flavonoids, polyphenols, glycosides, proteins, terpenoids, etc. Acetone extract of *J. adhatoda* leaves shows significant antibacterial activity with ZOI values of 14mm and 12mm with *Enterococcus faecalis* and *Bacillus subtilis* respectively. The methanol extract of stem of *T. cordifolia* showed the strongest DPPH radical scavenging activity with (IC_{50}=8.213µg/mL) close to standard ascorbic acid. The results revealed that *T. cordifolia* is a good source of antioxidants as compared to *J. adhatoda*. The total phenolic content was highest in the methanolic extract of *T. cordifolia* (46.463 mg GAE/g extract) while the *J. adhatoda* had lower values (31.167 mg GAE/g extract), the total flavonoid content was highest in methanol extract of *J. adhatoda* leaves (13.030 mg QE/g extract) while *T. cardifolia* had lower values (2.112 mg QE/g extract). Both plants are important ethnomedical values and have been used widely in folk medicine. However, research results showed that *J. adhatoda* contained a higher amount of flavonoids that's why it showed significant antimicrobial results and hence might be useful in cosmetics and drug formulation, while the stem of *T. cordifolia* shows high total phenolic content and would be better for respiratory problems, heart diseases, and cancer treatments.

Acknowledgment

Authors are grateful to Polyclinic Research Centre, Kathmandu, Nepal for providing antimicrobial results.

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