A | R | J
Amrit Research Journal December 2022, Vol. 3, Issue 1, 56-66
ISSN: 2738-9553
RESEARCH ARTICLE

GC-MS Profiling and Bioactivities of Essential Oil and Extracts of *Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm. Leaves from Kathmandu Valley, Nepal

Pooja Tandukar[1], Nabin Das[1], Ishwor Pathak[1], Daman Raj Gautam[1]*
[1] Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal
**Email: damanrajgautam@gmail.com*

(Received: August 18, 2022, Received in revised form: November 18, Accepted: December 4, 2022, Available Online)

Highlights

- Essential oil of *C. tamala* leaves was extracted by hydro-distillation, and the leaf extracts were prepared by the cold percolation method.
- GC-MS analysis showed the presence of 18 different compounds.
- Ethyl acetate extract showed the highest antioxidant activity.
- Methanol extract showed a higher TPC value, and ethyl acetate extract showed a higher TFC value than other extracts.
- Essential oil and chloroform extract showed considerable antimicrobial activities.

Abstract

The present work reports the phytoconstituents present in the essential oil and four different solvent extracts of Cinnamomum tamala leaves. Their antibacterial, antifungal, and antioxidant potential were also evaluated. The extraction of essential oil was performed by hydro-distillation using Clevenger apparatus and its chemical composition was identified by gas chromatography coupled with mass spectrometry (GC-MS). The leaf extracts were obtained by the cold percolation method. Linalool and cinnamaldehyde (E) were major compounds in the oil. Phytochemical screening of the extracts revealed the presence of terpenoid, glycoside, tannin, reducing sugar, polyphenol, saponin, flavonoid, and alkaloid. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified using Folin-Ciocalteu and Aluminium chloride colorimetric assay, respectively. The methanol extract (98.36 mg GAE/g) and ethyl acetate extract (90.44 mg GAE/g) showed higher TPC values. Similarly, the TFC value of ethyl acetate extract was higher (478.78 mg QE/ g) than the other extracts. The antioxidant activity of extracts was assessed by using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay in which the ethyl acetate extract showed high antioxidant efficacy. The essential oil and chloroform extract showed antifungal activity against Candida albicans, while only the oil showed activity against Shigella dysenteriae.

Keywords: *Cinnamomum tamala*, *antimicrobial, antioxidant, flavonoid content, phenolic content*

Introduction

Medicinal plants have been used as herbal medicine in the treatment of various human diseases for thousands of years. The practice of using plant resources in medicines varies according to tradition, climatic conditions, and the vegetation type of the place [1]. Nepal is considered as one of the richest countries, in terms of indigenous traditional knowledge, due to its geographical diversities and many ethnic communities [2]. Medicinal plants are also used in the Ayurveda system for the preparation of various

*Corresponding author

ayurvedic formulations. Ayurveda remains dominant compared to modern medicine, particularly for the treatment of a variety of chronic disease conditions [3]. As we enter a new millennium, the emergence of drug-resistant strains of microorganisms is increasing. Many premature deaths occur due to infectious diseases. A feasible way to combat the problem of microbial resistance is finding new chemicals, specifically herbals, for the development of new drugs [4]. Hence, the identification and characterization of medicinal plants are gaining importance as they are easily available and have non-toxic nature [5].

Plants constitute different phytochemicals, which are biologically active compounds, also called secondary metabolites. These include alkaloids, flavonoids, terpenoids, glycosides, phenolics, and saponins. These chemicals bear a variety of properties like antibacterial, antifungal, antiviral, antihelminthic, anticancer, sedative, laxative, cardiotonic, diuretic, and antioxidant [6, 7]. The ability to inhibit the growth of pathogenic microorganisms, without harming the host, demonstrates their potential application as therapeutic agents [8]. Herbs and spices have also been used as medicinal and food preservatives mainly in communities with inadequate health services and sanitation [10]. The utilization of essential oil is also very extensive and covers a wide range of human activities [11]. Essential oil is an aromatic liquid containing volatile chemical compounds obtained from different plant parts [12]. The fragrance oils contribute a major role in the commercial production of cosmetics, soaps, and perfumery items [13]. Essentials oils are added to many herbal products and they are known to be much effective against different fungi and bacteria [14].

Cinnamomum tamala is an evergreen tree that can grow up to 15-20 m in height and belongs to the Lauraceae family. The genus *Cinnamomum* is represented by about 350 species worldwide [15]. The leaves are generally known as Indian bay leaves (in English), Tejpat (in Nepali), Tejpatta (in Bhojpuri), or Tamalpatra (in Sanskrit). *C. tamala* is native to Nepal, India, China, Bhutan, and Bangladesh. In Nepal, it is found between 500-2000 m above sea level [16]. Leaves are olive green in colour, 10-15 cm long, 3-6 cm wide with three veins travelling from base to apex. They have shiny surfaces and are rarely elliptical but pointed towards the end. The leaves are thick, alternately placed, opposite, and short-stalked. Young leaves are light reddish. The taste of leaves is clove-like and the odour is pepper-like [17]. *C. tamala* is an important medicinal plant used as spices or flavouring additive in a variety of cuisines, savoury dishes, tea, and traditional foods [18]. Chemical constituents which have been widely reviewed and investigated are Eugenol and Cinnamaldehyde from leaves of *C. tamala* [19]. Eugenol is responsible for the anti-bacterial and anti-fungal activities of the leaves [20]. Since eugenol is an antioxidant, it can become an alternative to synthetic antioxidants used in food preservation [21]. The leaves have an antidiabetic effect which is mainly contributed by Cinnamaldehyde (3-phenyl-2-propenal), a potential antidiabetic agent [22]. The oil isolated from the leaves of *C. tamala* known as Tejpatta oil is medicinally used as a carminative, antiflatulent, diuretic and it has been reported to show antibacterial and hypoglycemic activity [21]. The essential oil also possesses antifungal and antioxidative properties [23]. It is well known that the variation in phytoconstituents of medicinal plants due to the topographical and environmental differences, in which the plants grew, affects their biological properties [24]. Herein, the present study was carried out to determine the presence of possible chemical groups and biological activities of essential oil and extracts of leaves of *C. tamala* from Budhanilkantha Municipality, Kathmandu, Nepal.

Materials and Methods

Collection of Plant Materials

The fresh leaves of *C. tamala* were obtained from Budhanilkantha-2, in May 2019. Identification of the plant was confirmed at the Department of Botany, Amrit Campus, Tribhuvan University, Kathmandu, Nepal.

Extraction of Essential Oil

Fresh leaves of *C. tamala* were washed with tap water and cut into small pieces. 100 grams of processed samples were placed into a round bottom flask along with distilled water. Hydro-distillation was performed in a Clevenger apparatus for about 3 hours to get light green volatile oil of characteristic odour. The essential oil was collected in a small glass vial and sealed properly. The process was repeated several times. The collected essential oil was stored in a refrigerator until further used.

GC-MS Analysis of Essential Oil

GC-MS analysis of the oil was performed at the Natural Product Research Laboratory, Department of Plant Resources,

Kathmandu, Nepal. A gas chromatography-mass spectrometer (RTX-5 MS) was used which had a column of dimension 60 m × 0.32 mm × 0.25 µm with helium as a carrier gas. The constituents of the oil were identified by comparing the mass spectra with those reported in NIST 2017 and FFNSC 1.3 libraries.

Determination of Chemical Parameters of Essential Oil

The acid value, saponification value, and iodine value of the essential oil of *C. tamala* leaves were determined by following the standard procedures [25].

Preparation of Plant Extracts

The collected leaves were washed in tap water, shade dried, and powdered by using an electric grinder. The cold percolation method was applied for the extraction of plant material. 200 grams of powdered leaves were soaked in about 800 mL hexane in a clean and dry container. After 7 days, the mixture was filtered through muslin cloth and re-filtered by passing through filter paper. The filtrate was concentrated under reduced pressure in a Rotary evaporator. Meanwhile, the residue obtained right after the filtration was completely air-dried. Then it was again soaked in chloroform, ethyl acetate, and methanol, sequentially to obtain respective extracts. Each time the above process was repeated and the concentrated filtrate was air-dried to obtain the blackish-green semisolid mass of the extract. The percentage yield of each extract was determined and all the extracts were stored in a refrigerator until used.

Phytochemical Analysis

The extracts were subjected to a preliminary phytochemical study for the detection of chemical constituents by following the standard protocols [26, 27].

Antioxidant Activity (DPPH assay)

The antioxidant activity of the extracts was determined by a DPPH assay. Different concentrations of test samples (20, 40, 60, 80, and 100 ppm) were prepared. 2 mL of test solution from each concentration was mixed with 2 mL of 0.2 mM DPPH solution. A control was prepared by mixing 2 mL methanol and 2 mL 0.2 mM DPPH solution. These samples were shaken well and kept in dark for 30 minutes. Their absorbance values were measured at 517 nm against methanol and DPPH as a blank solution. Ascorbic acid was used as a positive control. The measurement was performed in triplicates. The percentage of free radical scavenging activity was calculated using the following equation [28].

$$\% \text{ scavenging} = \left[\frac{(A_o - A_s)}{A_o}\right] \times 100$$

where, A_o = Absorbance of the control (methanol + DPPH solution) and A_s = Absorbance of sample solution. The IC_{50} value was calculated from the plotted graph of sample concentrations against the corresponding radical scavenging activity.

Total Phenolic Content

The total phenolic content of the extracts was estimated by using Folin-Ciocalteu reagent (FCR) [29]. Gallic acid solutions having concentrations 20, 40, 60, 80, and 100 ppm were prepared to make a standard solution. An aliquot of 1 mL gallic acid solution from each concentration was poured into test tubes, to which 5 mL of 10% FCR and 4 mL of 7% Na_2CO_3 were added. The blue-coloured mixture was shaken well and incubated for 30 minutes at 40 °C in a water bath. The absorbance of the solution was measured at 760 nm against a blank containing all reagents except gallic acid. All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve. The solution of all the extracts having concentrations 20, 40, 60, 80, and 100 ppm was prepared, and their absorbance values were measured following the same procedure as for gallic acid. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of extract in dry weight (mg/g).

Total Flavonoid Content

The total flavonoid content of the extracts was determined by Aluminium chloride colorimetric assay, according to the standard procedure [29]. Various concentrations of standard quercetin solutions of 20, 40, 60, 80, and 100 ppm were prepared. An aliquot of 1 mL quercetin solution from each concentration was poured into a test tube containing 4 mL of distilled water. At zero time, 0.3 mL of 5% $NaNO_2$ was added to the test tube. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was added and then after 6 minutes, 2

mL of 1M NaOH was added to the mixture. Immediately, 2.4 mL of distilled water was added and mixed thoroughly. Finally, the absorbance of the pink colour mixture was determined at 510 nm against a blank containing all the reagents except quercetin. The average absorbance values obtained at different concentrations of quercetin were used to plot the calibration curve. The solution of all the extracts having concentrations of 20, 40, 60, 80, and 100 ppm was prepared, and their absorbance values were measured following the same procedure as for quercetin. The total flavonoid content of the extracts was expressed as mg quercetin (QE) per gram of extract in dry weight (mg/g).

Antimicrobial Activity

The antibacterial assay was performed using the agar well diffusion method. Six strains of Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae*), four strains of Gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis*), and two fungal strains (*Candida albicans*, *Saccharomyces cerevisiae*) were used as test organisms. A mixture of the test sample and the appropriate solvent was homogenized by a vortex. The solvent used for extracts was dimethyl sulfoxide (DMSO), while that for the oil was 10% aqueous DMSO with 5% Polysorbate 80. Test organisms and sterilized nutrient broth were also homogenized. A sterile swab was used to evenly distribute bacterial or fungal culture over the suitable medium, Muller-Hinton Agar (MHA) for bacteria and Muller-Hinton Agar with Glucose and Methylene Blue (MHA, GMB) for fungi. The inoculated plates were allowed to dry for 15 minutes. Three wells for test samples and one well for the solvent as negative control were created in the inoculated plates where the test samples and the solvent were dispensed using micropipettes. The inoculated plates were then incubated for 18-24 hours at 35±2˚C for bacteria, and 24-48 hours at 25±2˚C for fungi. The zone of inhibition was determined by measuring the clear area around the well.

Results and Discussion

Percentage Yield

The essential oil was obtained from fresh leaves of *C. tamala* using the Clevenger apparatus, while four different solvents were used to get respective extracts from dry powdered leaves. The percentage yield of each extract and the essential oil obtained is given in Table 1. The methanol extract had the highest percentage yield among the four extracts.

Table 1. Percentage yield of the leaves extract and the essential oil of C. tamala

Chemical component	Percentage yield
Essential oil	0.5%
Hexane extract	2.06%
Chloroform extract	3.69%
Ethyl acetate extract	2.49%
Methanol extract	11.53%

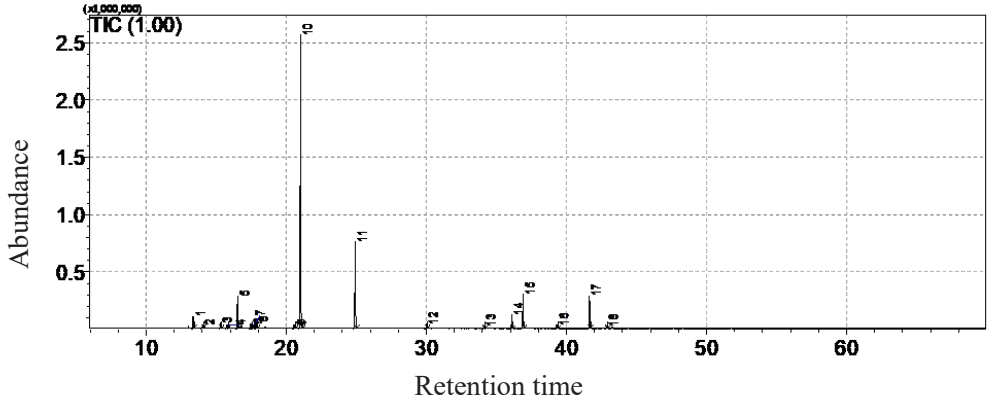

Fig 1. GC-MS chromatogram of essential oil of Cinnamomum tamala leaves

Chemical Analysis of Constituents of Essential Oil

The essential oil extracted from the leaves of *C. tamala* was analysed by GC-MS. The compounds identified in the oil are listed in Table 2 and the chromatogram of the oil is shown in Figure 1.

Table 2. Composition of the essential oil of C. tamala leaves based on GC-MS analysis

Peak	R.T.	Area%	Name of compounds	Class of compounds
1	13.352	2.15	Pinene <alpha->	monoterpene
2	14.041	0.60	Camphene	monoterpene
3	15.310	0.92	Pinene <beta->	monoterpene
4	15.797	0.53	Myrcene	monoterpene
5	16.527	5.44	Phellandrene <alpha->	monoterpene
6	17.473	0.86	Cymene <para->	monoterpene
7	17.678	0.64	Limonene	monoterpene
8	17.847	1.15	Eucalyptol	monoterpene
9	20.572	0.63	Terpinolene	monoterpene
10	21.032	54.01	Linalool	monoterpene
11	24.922	15.89	Cinnamaldehyde, (E)-	aldehyde
12	30.008	0.82	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,acetate,(1S-endo)-	ester
13	34.111	0.59	Spiro (6,6-dimethyl-2,3-diazobicyclo[3.1.0] hex-2-ene-4,1'-cyclopropane)	spiro cyclopropane
14	36.113	2.72	cis-beta-Farnesene	sesquiterpene
15	36.901	6.10	Cinnamyl acetate <(E)->	ester
16	39.332	0.76	Caryophyllene	sesquiterpene
17	41.643	5.70	Nerolidol	sesquiterpene
18	42.885	0.49	2,3-Diazabicyclo[2.2.1]hept-2-ene,5-ethenyl-4,7,7-trimethyl-, (1.alpha.,4.alpha.,5.beta.)-	

Eighteen compounds were identified representing 100% of the total oil. The major components were linalool (54.01%), cinnamaldehyde (E) (15.89%), cinnamyl acetate (E) (6.10%), nerolidol (5.70%), phellandrene (alpha) (5.44%), cis-beta-farnesene (2.72%), pinene (alpha) (2.15%) and eucalyptol (1.15%).

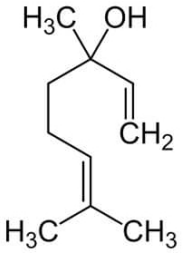

Fig 2. Chemical structure of linalool

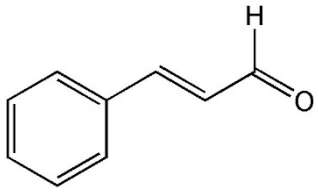

Fig 3. Chemical structure of cinnamaldehyde

Linalool is unsaturated monoterpene alcohol, which is a principal component of many essential oils [30]. It is used in the flavour and fragrance industries. It has antimicrobial, antioxidant, and anticancer properties [31]. Cinnamaldehyde is a phenylpropanoid that gives cinnamon its flavour and odour. Both compounds have been widely used as food additives. They are also the major active anti-inflammatory constituents in essential oil [32].

The chemical constituents present in the *C. tamala* oil samples collected from different places may vary. Tejpat oil from District

Plant Resources Office, Hetauda, Nepal, had linalool (41.39%) as the most significant component, the oil from Brindawan Botanical Garden, Hetauda, had eucalyptol (12.08%) as a major component, whereas the oil from Tistung Botanical Garden, Hetauda, had (E)-cinnamyl acetate as the highest component [33]. The major component of essential oils from the *C. tamala* leaves of Indian origin was reported to be cinnamaldehyde, of 44.898% [18] and 37.85% [10]. Likewise, the oil from Mizoram, Northeast India had linalool (67.6%) as major constituents [34]. The chemical composition of the essential oil can be considered to vary due to the differences in geographical regions, altitudes, seasons, and other climatic influences [35].

Determination of Chemical Parameters of Essential Oil

The acid value measures the presence of free fatty acids in the oil. Free fatty acids are considered a defect in the oil as they deteriorate the age and quality of the oil. The acid value of the essential oil of *C. tamala* leaves was found to be 0.76 which indicates that the oil has high storage life. In saponification, triglycerides of fatty acids are hydrolyzed with alkali to produce glycerol and alkali salts of fatty acids. This process is highly significant in the making of soap [12]. The saponification value of the essential oil of *C. tamala* leaves was found to be 300 mg KOH/g. It means 300 mg of KOH is required to saponify 1g of the oil. The iodine value gives an idea of the average degree of unsaturation of the oil. The higher the iodine value, the greater the number of C=C double bonds [12]. The iodine number of the essential oil of *C. tamala* leaves was found to be 36.2 g I_2/100g. It means 36.2 grams of iodine consumed by 100 grams of unsaturated oil which shows that the oil is unsaturated.

Qualitative Analysis of Phytochemicals

The phytochemical test result of *C. tamala* leaves extracts in the various solvent is given in the table 3 below.

Table 3. Phytochemical screening of the leaves extracts of C. tamala

Phytochemicals	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloids	-	-	+	-
Terpenoids	-	+	+	+
Coumarins	-	-	-	-
Flavonoids	+	+	+	+
Quinones	-	-	-	-
Polyphenols	+	+	+	+
Glycosides	-	-	-	+
Reducing sugars	-	+	-	-
Saponins	-	-	-	+
Tannins	+	+	-	+

Indications: '+' means presence and '-' means absence

The result revealed that flavonoids and polyphenols were present in all four solvent extracts. Alkaloid was found in ethyl acetate extract only. Similarly, reducing sugar was only found in chloroform extract. Glycosides and saponins were present in methanol extract. Terpenoid was absent in hexane extract, tannin was absent in ethyl acetate extract, while coumarins and quinones were absent in all the extracts. On comparing with other studies, the methanolic extract of *C. tamala* leaves contained tannin, alkaloid, terpenoid, phenol, flavonoid, saponin, and reducing sugar, which possess direct or indirect correlation with therapeutic efficacy against various diseases [4, 8, 20, 36]. Likewise, it was found that the crude methanolic extract of *C. tamala* leaves was rich in alkaloids, reducing sugar, tannin, amino acids, glycosides, and steroid [22].

Antioxidant Activity

The antioxidant activity of *C. tamala* leaves extracts was evaluated using the DPPH assay. Ascorbic acid was taken as standard. Table 4 shows the absorbance values, % radical scavenging activity, and IC_{50} values of extracts and ascorbic acid taken at different concentrations. Figure 4 represents a plot between sample concentrations and % free radical scavenging activity.

Table 4. Absorbance values, % radical scavenging, and IC_{50} values of samples at different concentration

Concentration (ppm)	Absorbance values					% radical scavenging				
	HE	**CH**	**EA**	**ME**	**AA**	**HE**	**CH**	**EA**	**ME**	**AA**
0	0	0	0	0	0	0	0	0	0	0
20	1.022	0.915	0.978	0.982	0.859	3.40	13.51	7.56	7.18	18.81
40	1.005	0.908	0.886	0.968	0.751	5.00	14.17	16.25	8.50	29.01
60	0.989	0.888	0.802	0.919	0.516	6.52	16.06	24.19	13.14	51.22
80	0.979	0.853	0.774	0.872	0.157	7.46	19.37	26.84	17.58	85.16
100	0.938	0.841	0.646	0.834	0.080	11.34	20.51	28.94	21.17	92.43
				IC_{50} values (ppm)		491.14	256.90	133.41	241.4	53.98

Indications: HE: Hexane extract, CH: Chloroform extract, EA: Ethyl acetate extract, ME: methanol extract, AA: Ascorbic acid
Absorbance of control (methanol + DPPH solution) = 1.058

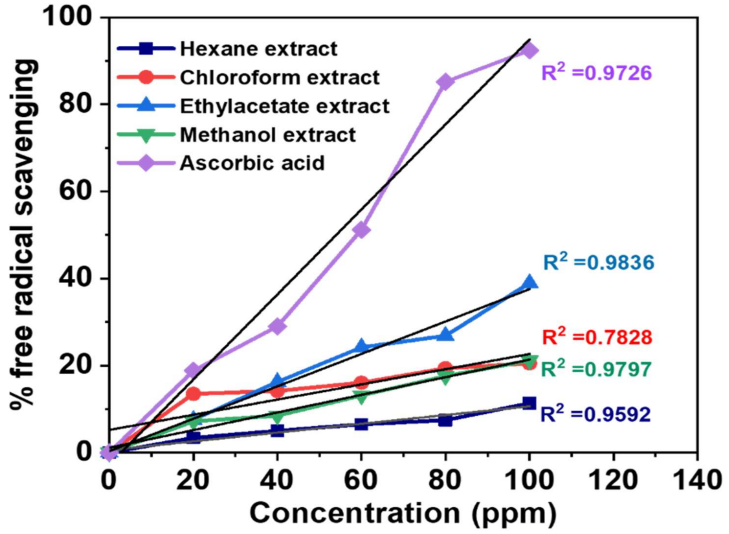

Fig 4. Comparison of % radical scavenging between ascorbic acid and different extracts of C. tamala leaves

Antioxidant activity is inversely proportional to the IC_{50} value, which that means, a lower value of IC_{50} indicates high antioxidant potential. Compared to the standard ascorbic acid which had an IC_{50} value of 53.98 µg/mL, the ethyl acetate extract showed high antioxidant activity with IC_{50} 133.41 µg/mL. The methanol extract and chloroform extract showed the IC_{50} values of 241.4 µg/mL and 256.90 µg/mL respectively. The hexane extract showed low antioxidant activity with IC_{50} 491.14 µg/mL. This work is also supported by a previous report which showed ethyl acetate extract had better reducing activity [23]. In similar studies, the methanolic extract also showed appreciable antioxidant activity [21, 37, 38]. The antioxidant property of plant extracts is generally associated with the quantitative abundance of phenolics and flavonoids in the plant [35].

Total Phenolic and Flavonoid Contents

The total phenolic content of *C. tamala* leaves extracts was determined by using Folin-Ciocalteu colorimetric method. Gallic acid was used as a standard. The TPC of different extracts was calculated from the calibration curve of gallic acid by regression equation, followed by the formula C= cV/m and expressed as mg gallic acid equivalents (GAE) per gram of extract in dry weight (mg/g). Similarly, Aluminium choride colorimetric assay was used for the estimation of total flavonoid content in the leaves extracts. Quercetin was used as a standard. The TFC of different extracts was calculated from the calibration curve of quercetin by regression equation, followed by the formula C= cV/m and expressed as mg quercetin (QE) per gram of extract in dry weight (mg/g). The results obtained are given in Table 5.

Table 5. Total phenolic and total flavonoid contents of *C. tamala* extracts

Extract	TPC (mg GAE/ g)	TFC (mg QE/ g)
Hexane	21.75	343.56
Chloroform	44.6	388.34
Ethyl acetate	90.44	478.78
Methanol	98.36	393.56

It was found that the total phenolic content was highest in methanolic extract (98.36 mg GAE/ g) and lowest in hexane extract (21.75 mg GAE/ g). Similarly, the highest amount of flavonoid was detected in ethyl acetate extract (478.78 mg QE/ g) while the lowest amount was detected in hexane extract (343.56 mg QE/ g). The literature also reported sufficient amount of total phenolic and total flavonoid contents [20, 22, 36].

Antimicrobial Activity

Agar well diffusion method was used to determine the antimicrobial activity which was measured in the form of a zone of inhibition (ZOI). The results are given in Tables 6 and 7.

Table 6. Antibacterial activity of essential oil, chloroform extract and methanol extract of *C. tamala* leaves

Bacterial strains	Diameter of Zone of Inhibition (mm)			
	Positive control Chloramphenicol (60 µg.mL^{-1})	***C. tamala* Essential oil**	***C. tamala* Chloroform extract**	***C. tamala* Methanol extract**
Gram negative bacteria				
Escherichia coli	20.86	0	0	0
Klebsiella pneumoniae	12.28	0	0	0
Proteus vulgaris	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0
Salmonella typhi	27.44	0	0	0
Shigella dysenteriae	28.99	8	0	0
Gram positive bacteria				
Bacillus subtilis	26.58	0	0	0
Enterococcus faecalis	20.86	0	0	0
Staphylococcus aureus	28.4	0	0	0
Staphylococcus epidermidis	31.47	0	0	0

Table 7. Antifungal activity of essential oil, chloroform extract, and methanol extract of *C. tamala* leaves

Fungal strains	**Diameter of Zone of Inhibition (mm)**			
	Positive control Clotrimazole (200 µg.mL^{-1})	***C. tamala* Essential oil**	***C. tamala* Chloroform extract**	***C. tamala* Methanol extract**
Candida albicans	32.33	20	14.61	0
Saccharomyces cerevisiae	24.25	0	0	0

The essential oil of *C. tamala* leaves exhibited antibacterial activity against only *S. dysenteriae* with a ZOI value of 8 mm. The chloroform and methanol extracts did not show antibacterial activity against any of the test bacteria. The essential oil and chloroform extract showed antifungal activity against *C. albicans* with ZOI values of 20 and 14.61 mm respectively, but inactive against *S. cerevisiae*. The methanol extract showed no activity against any test fungi. However, the previous studies by several authors have shown the oil and extract to be effective against most of the bacteria and fungi listed in Tables 6 and 7 [8, 10, 20,

33, 39]. These differences may be due to the altitude variation, the time and the method of sample collection, and the chemical grades in the lab.

Conclusions

The essential oil of *C. tamala* leaves was obtained by using a Clevenger apparatus. GC-MS analysis of the essential oil showed the presence of eighteen compounds, where linalool was the major component. The antimicrobial activity of the oil and the leaves extracts was carried out against ten bacteria and two fungi, using the agar well diffusion method. The oil showed activities against *S. dysenteriae* and *C. albicans*. Among the four extracts prepared in hexane, chloroform, ethyl acetate and methanol solvents, only chloroform extract showed antifungal activity against *C. albicans*. The extracts also exhibited various degrees of antioxidant activities in the DPPH assay. The bioactivities of the extracts could be attributed to the presence of different phytochemicals, mainly phenolics and flavonoids as their contents were relatively higher. The results from the present work showed that *C. tamala* could be a source of natural antioxidants in herbal medicine for various ailments. It also acts as antimicrobials against bacteria and fungi. Further research is required to confirm many of the therapeutic claims for *C. tamala*. As it is one of the popular spices, the demand for this plant is very high in the national as well as international market, so expansion of cultivation is also recommended.

Acknowledgements

The authors are thankful to the Department of Chemistry, Amrit Campus, Tribhuvan University for providing the laboratory facilities and are grateful to the Department of Plant Resources, Thapathali, Kathmandu for the antimicrobial test and GC-MS analysis.

References

1. S. Shah, and D. Lamichhane, Documentation of Indigenous Knowledge on Plants used by Tamang Community of Kavrepalanchok District, Central Nepal, *Journal of Plant Resources* (2017), *15*(1), 45-51.
2. S. Sharma, R. Bajracharya, and B. Sitaula, Indigenous Technology Knowledge in Nepal- a Review, *Indian Journal of Traditional Knowledge* (2009), *8*(4), 569-576.
3. B. Patwardhan, A.D.B. Vaidya, and M. Chorghade, Ayurveda and Natural Products Drug Discovery, *Current Science* (2004), *86*(6), 789-799.
4. A. Jain, M. Dubey, A. Gupta, S. Mahajan, and H.S. Chaudhari, Antimicrobial Activity of *Cinnamomum tamala* (Tejpat) against some Bacterial and Fungal Pathogens, *Journal of Pharmacy Research* (2011), *4*(11), 3975-3977.
5. K.V. Khajapeer, P.P. Krishna, and R. Baskaran, GC MS and Elemental Analysis of *Cinnamomum tamala*, *International Journal of Pharmacy and Pharmaceutical Sciences* (2015), *7*(8), 398-402.
6. C.M. Gurmachhan, U. Tandukar, N. Shrestha, P.B. Lakhey, and C.P. Pokherel, Antibacterial and Phytochemical Studies of Bark Extract of *Berberis Asiatica* Roxb. ex. DC. and *Myrica esculenta* Buch.-Ham ex. D. Don., *International Journal of Plant Resources* (2019), *17*(1), 139-146.
7. S.K. Kalauni, M. Niraula, P. Thapa, and I. Pathak, Comparative Studies on Antioxidant Activity of Ten Medicinal Plants Collected from the Ilam District of Nepal, *Nepal Journal of Science and Technology* (2021), *20*(1), 136-145.
8. W. Hassan, S.N.Z. Kazmi, H. Noreen, A. Riaz, and B. Zaman, Antimicrobial Activity of *Cinnamomum tamala* Leaves, *Journal of Nutritional Disorders & Therapy* (2016), *6*(2), 1-5.
9. R. Marahatha, K. Gyawali, K. Sharma, N. Gyawali, P. Tandan, A. Adhikari, G. Timilsina, G. Lamichhane, N. Parajuli, S. Bhattarai, A. Acharya, I. Pathak, and H.P. Devkota, Pharmacologic Activities of Phytosteroids in Inflammatory Diseases: Mechanism of Action and Therapeutic Potentials, *Phytotherapy Research* (2021), *35*, 5103-5124.
10. M. Mohan, S.Z. Haider, A. Sharma, R. Seth, and M. Sharma, Antimicrobial Activity and Composition of the Volatiles of *Cinnamomum tamala* Nees. and *Murraya koenigii* (L.) Spreng. from Utarakhand (India), *Asian Pacific Journal of Tropical Disease* (2012), *2*, S324-S327.

11. R. Ranjitkar, D.P. Bhandari, and L. Bhandari, Acute Toxicity Test of Ten Commercial Essential Oils of Nepalese Origin, *Journal of Plant Resources* (2019), *17*(1), 82-85.
12. R. Timilsina, P. Tandukar, and I. Pathak, Biological and Chemical Studies of Essential Oil and Extracts of Rhizome of *Acorus calamus* Linn., *Journal of Nepal Chemical Society* (2022), *43*(1), 35-42.
13. A. Kumar, Physico-Chemical and Natural Products Investigations of Essential Oil from the Rhizomes of *Kaempferia galanga* L. Der Chemica Sinica (2014), *5(2),* 91-94.
14. M. Irshad, M.A. Subhani, S. Ali, and A. Hussain, *Biological Importance of Essential Oils, Essential Oils – Oils of Nature*, El-Shemy, H.A., IntechOpen: London, UK, 2020.
15. U. Chakraborty, and H. Das, Antidiabetic and Antioxidant Activities of *Cinnamomum tamala* Leaf Extracts in STZ-Treated Diabetic Rats, *Global Journal of Biotechnology & Biochemistry* (2010), *5*(1), 12-18.
16. M.S. Thapa, and B. Khatri Chhetri, Management Practice of *Cinnamomum tamala* in Eladi VDC of Syangia, Nepal, *Himalayan Biodiversity* (2017), *5*, 57-65.
17. S. Tiwari, and S. Talreja, Importance of *Cinnamomum tamala* in the Treatment of Various Diseases, *Pharmacognosy Journal* (2020), *12*(6), 1792-1796.
18. S. Kumar, N. Vasudeva, and S. Sharma, GC-MS Analysis and Screening of Antidiabetic, Antioxidant and Hypolipidemi Potential of *Cinnamomum tamala* Oil in Streptozotocin Induced Diabetes Mellitus in Rats, *Cardiovascular Diabetology* (2012), *11*(95), 1-11.
19. H. Kharkwal, S. Gill, and P. Panthari, Phytochemical Investigation of High Altitude Medicinal Plants *Cinnamomum tamala* (Buch-Ham) Nees and Eberm and *Rhododendron arboretum* Smith, *American Journal of Phytomedicine and Clinical Therapeutics* (2015), *3*(6), 512-528.
20. S. Dandapat, M. Kumar, and M.P. Sinha, Assessment of Bioactivity of *Cinnamomum tamala* (Buch-Ham.), *Turkish Journal of Agriculture- Food Science and Technology* (2015), *3*(3), 121-125.
21. R. Sudan, M. Bhagat, S. Gupta, Chitrarakha, and T. Devi, Comparative Analysis of Cytotoxic and Antioxidant Potential of Edible *Cinnamomum verum* (bark) and *Cinnamomum tamala* (Indian Bay Leaf), *Free Radicals and Antioxidants* (2013), *3*(2), S70-S73.
22. J. Ahmed, N. Sultana, S.M.R. Dewan, M.N. Amin, and S.M.N. Uddin, Determination of Chemical Groups and Investigation of Anthelmintic, Cytotoxic and Antibacterial Activities of Leaves of *Cinnamomum tamala* (Family: Lauraceae), *International Journal of Pharmamedix India* (2013), *1*(2), 222-232.
23. A.K. Pandey, A.K. Mishra, and A. Mishra, Antifungal and Antioxidative Potential of Oil and Extracts Derived from Leaves of Indian Spice Plant *Cinnamomum tamala*, *Cellular & Molecular Biology* (2012), *58*(1), 142-147.
24. I. Pathak, R. Budhathoki, N. Yadav, M. Niraula, and S.K. Kalauni, Phytochemical Screening, Cytotoxic and Antioxidant Activity of *Alternathera sessilis* and *Moringa oleifera*, *Amrit Research Journal* (2020), *1*(1), 65-71.
25. N.M. Khadka, S.D. Gautam, and P.N. Yadav, *A Core Experimental Chemistry*, 5th Edition Reprint, Heritage Publishers and Distributors Pvt. Ltd., Bhotahity, Kathmandu, Nepal, 2018.
26. M.N. Abdullahi, N. Ilyas, and H. Ibrahim, Evaluation of Phytochemical Screening and Analgesic Activity of Aqueous Extract of the Leaves of *Microtrichia perotitii* DC (Asteraceae) in Mice using Hotplate Method, *Medicinal Plant Research* (2013), *3*(5), 37-43.
27. I. Ciulei, *Methodology for the Analysis of Vegetable Drugs*, Chemical Industries Branch, Division of Industrial Operations, UNIDO, Romania, 1994, 21-97.
28. K.R. Sharma, S.K. Kalauni, and S. Awale, Antioxidant, Phytotoxic and Antimicrobial Activities of Methanolic Extract of *Bauhinia variegate* Barks, *Journal of Institute of Science and Technology* (2015), *20*(2), 37-41.
29. A.L. Waterhouse, Determination of Total Phenolics, *Current Protocols in Food Analytical Chemistry*, 6(1) (2002) 1-1.
30. I. Pathak, S. Rokaha, and K. Bajracharya, Phytoconstituents and Biological Activities of *Zanthoxylum armatum* Fruit Extract, *Journal of Nepal Chemical Society* (2021), *42*(1), 125-131.

31. G.P.P. Kamatou, and A.M. Viljoen, Linalool- a Review of a Biologically Active Compound of Commercial Importance, *Natural Product Communications* (2008), *3*(7), 1183-1192.
32. S.C. Lee, S.Y. Wang, C.C. Li, and C.T. Liu, Anti-inflammatory Effect of Cinnamaldehyde and Linalool from the leaf Essential Oil of *Cinnamomum osmophloeum* Kanehira in Endotoxin-Induced Mice, *Journal of Food and Drug Analysis* (2018), *26*, 211-220.
33. M. Rana, P.B. Lakhey, T.D. Bhatt, S. Khadgi, K. Paudel, A.K. Adhikari, M.R. Bhattarai, and S. Upadhyay, GCMS Qualitative Analysis and Antimicrobial Activity of Essential Oils of *Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm. (Tejpat) Leaves Collected from Different Parts of Makwanpur District, Nepal, *Journal of Plant Resources* (2017), *15*(1), 73-80.
34. G. Tewari, A. Rani, C. Pande, and K. Patni, A Review on Aroma Profile of *Cinnamomum* Species in North & North East India, *World Journal of Pharmaceutical Research* (2017), *6*(11), 200-221.
35. I. Pathak, and M. Niraula, Assessment of Total Phenolic, Flavonoid Content and Antioxidant Activity of *Ocimum sanctum Linn*. *Journal of Nepal Chemical Society* (2019), 48, 30-35.
36. A.R. Juvekar, D.R. Thanekar, and J.B. Dhodi, Evaluation of In Vitro Cytotoxic Activity of Petroleum Ether, Methanol and Aqueous Extracts of Indian Bay Leaf, *Cinnamomum tamala* (Buch.-Ham) T. Nees & Eberm on Cancer Cells, *World Journal of Pharmaceutical Sciences* (2013), *3*(1), 519-533.
37. S.K. Kalauni, R. Maharjan, K. Khadayat, M. Niraula, P. Thapa, and I. Pathak, Different Crude Extracts of *Cinnamomum tamala* with Antioxidant and Antibacterial Capabilities, *Amrit Research Journal* (2021), *2*(1), 68-74.
38. A.T.M. Zafrul Azam, R. Al-Mamum, A. Hamid, M.K. Islam, and J.A. Chowdhury, Lipid Lowering Activity and Free Radical Scavenging Effect of *Cinnamomum tamala* (Fam: *Lauraceae*), *International Journal of Natural Sciences* (2011), *1*(4), 93-96.
39. I.P.S. Kapoor, B. Singh, G. Singh, V. Isidorov, and L. Szczepaniak, Chemistry, Antimicrobial and Antioxidant Potentials of *Cinnamomum tamala* Nees & Eberm. (Tejpat) Essential Oil and Oleoresins, *Natural Product Radiance* (2009), *8*(2), 106-116.