

Phytochemical screening, isolation and estimation of total phenolic contents & total flavonoids contents of some medicinal plants

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ABSTRACT

The current study was based on the phytochemical screening, isolation and estimation of total phenolic contents & total flavonoids contents of some medicinal plants i.e., *Caesulia axillaris* Roxb, *Clerodendrum Viscosum* Ventenat and *Allium stracheyi* Baker. The Fresh leaves of *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat, *Allium stracheyi* Baker. were collected and washed making dust-free and dried under shade. The dried leaves were rendered into coarse powders and then finally into fine ones. The powders of each plant were weighed and soaked in aqueous, methanolic and hydroalcoholic solvent (1:1), separately for 15 days with frequent stirrings. After preliminary phytochemical screening, various herbal extracts were estimated for total phenolic content and total flavonoid content. Isolation of bioactive molecules was done using TLC and HPTLC analysis. In HPTLC analysis, leaves extract of *Caesulia axillaris* Roxb showed the expected compounds as Phenylpropene, and *Clerodendrum viscosum* Ventenat reported as phenolic compounds. Moreover, leaves extract of *Allium stracheyi* Baker. demonstrated Presence of flavonoids and phenol when observed in n-Hexane: ethyl acetate: methanol (70:15:15). It concluded that all three plants i.e., *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat and *Allium stracheyi* Baker. taken in the study demonstrated as a rich source of phytochemicals including alkaloids, cardiac glycosides, tannins, saponins, coumarins, steroids and flavonoids in descending order of hydroalcoholic>methanolic>aqueous extracts. In further studies, bioactive constituents might be isolated from the *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat and *Allium stracheyi* Baker., which could be isolated in the management of various diseases.

Keywords: *Caesulia axillaris* Roxb, *Clerodendrum Viscosum* Ventenat, *Allium stracheyi* Baker., total phenolic content, total flavonoid content.

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INTRODUCTION

Caesulia axillaris Roxb. flourishes in wetland ecosystems i.e., marshes, riverbanks, and other saturated environments. It is essential to the local ecology and well suited to these environments [1][2]. Along the stalk, the leaves are arranged in alternating order. Their lanceolate to elliptic shape makes photosynthesis more efficient. The majority of leaf edges are entire, although they can occasionally show slight serration, which adds to their variety. In addition to providing shelter and food for a variety of species, including pollinators like bees and butterflies, *C. axillaris* Roxb. improves the overall health of the wetland environment by stabilizing the soil and helping to purify the water [3].



Fig 1. *Caesulia axillaris* Roxb herb

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Taxonomy

Kingdom - Plantae
Order - Asterales
Class - Magnoliopsida
Family - Asteraceae
Genus - *Caesulia*
Species - *axillaris*

C. axillaris Roxb is rich in a number of bioactive compounds that improve its pharmacological characteristics. The plant's antioxidant, anti-inflammatory, antibacterial, analgesic, and gastroprotective qualities are attributed to a variety of components, including phenolic compounds, flavonoids, alkaloids, terpenoids, saponins, and essential oils. The potential therapeutic benefits of *C. axillaris* Roxb are being revealed by ongoing research on these bioactive compounds [4][5]. Limonene and γ -Asarone make up the majority of it.

The blossoming shrub *Clerodendrum Viscosum* Ventenat is known for its ugly foliage. The straight, unbranched stem is 0.5–4 m (1.6–13.1 ft) tall and has round leaves that are 15 cm (5.9 in) in diameter. The leaves are simple, opposite, elliptic, wide elliptic, ovate, or elongated oval, with a dentate edge, measuring 3.5–20 cm (1.4–7.9 in) in width and 6–25 cm (2.4–9.8 in) in length. Both surfaces are sparsely covered with villous-pubescent hairs. The inflorescence is a terminal cyme with few flowers that is peduncled [6][7].



Fig 2. *Clerodendrum Viscosum* Ventenat shrub

Taxonomy

Kingdom - Plantae
Order - Lamiales
Family - Lamiaceae
Genus - *Clerodendrum*
Species - *Viscosum* Vent.

C. viscosum Ventenat contains a number of bioactive phytochemicals. Because of their different spatial arrangement, the phytochemical profiles of the leaf, root, and flower show different bioactivities in the seed and stem. Flavonoids, terpenoids, steroids, and alkaloids are the main bioactive compounds that have been extracted from *C. viscosum* Ventenat, according to numerous research. Numerous bioactive compounds with notable pharmacological efficacy were found in many parts of *C. viscosum* Ventenat, according to thorough research [8][9]. Gallic acid, methyl gallate, tannic acid, ellagic acid, reserpine, limonene, α -pinene, β -pinene, and p -cymene are the main constituents of *C. viscosum* Ventenat leaves.

Allium stracheyi Baker. is a perennial herb that can grow up to 35 centimeters in height. The heads are globose, the pedicel is shorter than the flower, and the tepals are dark pink-reddish; the stem is glabrous, leafy at the base, and has thin, linear, flattened leaves [10]. In the Garhwal region, it is known locally as Jamboo, Dhungar, and Pharan. At elevations between 2500 and 3625 meters, this species can be found in Jammu Kashmir, Himachal Pradesh, Uttarakhand, Nepal, and Pakistan [11].

Taxonomy

Kingdom - Plantae
Order - Asparagales
Class - Liliopsida
Family - Liliaceae
Genus - *Allium*
Species - *stracheyi* Baker.



a) *Allium stracheyi* Baker. herb



Fig 3. Diverse parts of *Allium stracheyi* Baker.

Herbal medicine constitutes a complex system of formulations. Its formulations for medical use comprise several types of secondary metabolites or bioactive substances. Diverse chemical and analytical methodologies facilitate the comprehension of quality control and chemical ingredients in herbal pharmaceuticals. *Allium* species have been documented to contain a variety of physiologically active chemicals, including phenolic acids, flavonoids, thio-sulfinates, alkaloids, fixed oils, phytosterols, sulfur-containing compounds, and others [12][13].

A. stracheyi Baker. has sulfur-rich compounds having the antioxidant, anti-inflammatory, and antibacterial properties. Compounds high in sulphur have been documented to reduce blood cholesterol [14]. The current study was based on the phytochemical screening, isolation and estimation of total phenolic contents & total flavonoids contents of some medicinal

plants i.e., *Caesulia axillaris* Roxb, *Clerodendrum Viscosum* Ventenat and *Allium stracheyi* Baker.

MATERIALS AND METHODS

Experimental requirements

Fresh leaves of *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat, *Allium stracheyi* Baker. methanol, ethanol, distilled water, rotatory evaporator and weighing machine.

Collection, authentication, and preparation of extract

The Fresh leaves of *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat, *Allium stracheyi* Baker. were collected from UP East region and authenticated by a Botanist Dr Vijay Kumar Sinhal, Department of Plant Science, MJP Rohilkhand University Bareilly, UP India, with the ref. no. MJPRU/PS/2024/01. The leaves of *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat, *Allium stracheyi* Baker. were washed to make dust-free and dried under shade. The leaves were rendered into coarse powder and further in fine ones. The powders of each plant were weighed and soaked in aqueous, methanolic and hydroalcoholic solvent (1:1), separately for 15 days with frequent stirrings. The obtained slurry was kept for drying under partial vacuum using a rotatory evaporator [15].

Phytochemical screening [16][17].

Detection of Alkaloids

Extracts were dissolved individually in dilute HCl and filtered and then tested for following qualitative tests.

Mayer's Test: Filtrate was treated with Mayer's reagent. Presence of a yellowish precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent. It is confirmed by the formation of reddish-brown precipitate.

Hager's Test: Filtrates were treated with Hager's Reagent. Alkaloid presence is confirmed by the formation of yellow ppt.

Estimation of Glycosides

Fehling's test: Fehling's solutions A & B are heated for one minute with dilution of distilled water. Eight drops of plant extract were included into this translucent blue solution. The mixture was subsequently blended with 1 ml of Fehling's solution and cooked for 5 minutes in a water bath. Brick red precipitation signifies the presence of glycoside content.

Estimation of Saponins

Approx. 2 g of the plant extract were combined with 10 ml of distilled water and agitated vigorously to achieve a stable, persistent foam. The formation of foam signifies the presence of saponins.

Estimation of Tannins

Ferric chloride test: 0.5 g of the dried powdered sample were subjected to boiling in 20 ml of water within a test tube and subsequently filtered. A few drops of 0.1% FeCl₃ are added and monitored for a brownish green-black colouration.

Lead acetate test: 2 ml of plant extract was mixed with 2 ml of distilled water. 0.01 g of lead acetate is introduced into the mixed solution and thoroughly agitated. The emergence of white turbidity and precipitate signifies the existence of tannins.

Estimation of Flavonoids

NaOH test: A minimal quantity of extract was subjected to treatment with aqueous NaOH and HCl, and monitored for the development of a yellow-orange hue.

H₂SO₄ test: A portion of the extract was treated with concentrated sulphuric acid and monitored for the development of an orange hue.

Estimation of Terpenoids

5 ml of the aqueous plant extract was mixed with 2.0 ml of chloroform, subsequently evaporated using a water bath, and then heated with 3 ml of concentrated H₂SO₄. As terpenoids developed, a grey hue manifested.

Estimation of Steroids

2 ml of chloroform and concentrated sulphuric acid were combined with 5 ml of aqueous plant crude extract. A crimson hue emerged in the bottom chloroform layer, signifying the presence of steroids.

Test for Reducing Sugars and Carbohydrates

Molisch test

Add few drops of α -naphthol solution in alcohol were added to 2-3 ml of each solvent extract, mixed, and then introduced concentrated H₂SO₄ around the walls of the test tube. Violet ring at the interface of two liquids appears.

Fehling's test

It was employed to detect reducing sugars. Prepared a volume of 500 ml by dissolving 34.6 g of copper sulphate in distilled water (solution A). 50 g of sodium hydroxide and 17.3 g of potassium sodium tartrate were dissolved in distilled water to achieve a total volume of 50 ml (Solution B). Then both solutions were amalgamated in equal volumes. Fehling's A and B solutions in a 1 ml combination were heated to boiling for one minute and then incorporated the test solution in an equivalent quantity. Heated in a vessel of boiling water for 5 to 10 minutes. A golden colour was observed initially, followed by a brick red hue.

Determination of total phenolic content

The Folin-Ciocalteu reagent was employed to analyse the total phenolic content [18]. Folin-Ciocalteu reagent (0.5 ml) was mixed with 0.5 ml of each extract. Before adding 2 ml of a 7.5% sodium carbonate solution and adjusting the volume up to 8 ml with water, the solution was kept at 25°C for five to eight minutes. The absorbance was measured at 725 nm after two hours. The calibration curve's standard was gallic acid. Milligrams of gallic acid equivalents per gram of sample (mg/g) were used to measure the total phenolic content.

Determination of total flavonoids content

The total flavonoid content was quantified using a colorimetric technique [19]. 4ml of water was mixed with 100µl of each extract. 0.3 cc of 5% sodium nitrite was then added. Five minutes later, 0.3ml, 10% aluminum chloride were added. Two milliliters of 1 M sodium hydroxide were added to the mixture after six minutes. 3.3 cc of filtered water was quickly added to dilute the mixture, and it was thoroughly mixed. At 510 nm, the absorbance was measured in comparison to a blank sample. The calibration curve was based on catechin. Each extract's total flavonoid concentration was measured in mg of catechin equivalents per gm of sample.

Isolation of bioactive compounds [20]

Thin Layer Chromatography Analysis

Using a fine capillary, each herbal extract was placed as dots to the precoated TLC plate following its dissolution in the appropriate solvents. Identifying markings were located on the plate's upper surface. Rectangular glass containers were used for the chromatographic test. To prevent insufficient chamber saturation, a smooth sheet of filter paper was inserted into the TLC chamber; submerged in developing solvent. After applying anisaldehyde-sulfuric acid to the plate, it was heated for five minutes at 115°C. Chloroform, ethyl acetate, and acetone were used in a 7:1.5:1.5 ratio as the solvent system. Spot count, color, and R_f values were measured after the plates were prepared and allowed to air dry.

HPTLC Analysis

In HPTLC the stationary phase is generally a thin coating of silica gel or other adsorbents. The selection of stationary phase is contingent upon the characteristics of the sample constituents. The mobile phase may consist of a solvent combination, contingent upon the solubility of the analytes. The typical mobile phase comprises a combination of polar and non-polar solvents, facilitating effective component separation.

Sample preparation and Chromatographic separation

To prepare the sample, dissolve the plant extract in methanol. To get rid of solid materials, filtration was used. Using a micropipette, little amounts of the material were applied to the TLC plate. As much as feasible, the sample application was concentrated on a single area and attempted to be consistent.

In order to promote the mobile phase's ascent through capillary action, the plate was put in a growth chamber. The components of the sample migrated at different velocities depending on how they interacted with the stationary phase, causing separation. Following growth, the plate was dried out and subjected to UV light or staining techniques for colorimetric assessment.

RESULTS AND DISCUSSION

Percentage yield

The aqueous, methanolic and hydroalcoholic leaves extract of *Caesulia axillaris* Roxb showed the percentage yield as 5.32%, 6.68%, 8.42%, respectively. *Clerodendrum viscosum* Ventenat showed the percentage yield as 7.16%, 9.54%, 10.34%, in an aqueous, methanolic and hydroalcoholic fractions. The

percentage yield was observed as 6.38%, 7.92%, 9.64%, in aqueous, methanolic and hydroalcoholic leaves extract of *Allium stracheyi* Baker.

Table 1. Percentage yield of medicinal plants

Plant extract		Percentage yield (%)
<i>Caesulia axillaris</i> Roxb	Aqueous	5.32
	Methanolic	6.68
	Hydroalcoholic	8.42
<i>Clerodendrum viscosum</i> Ventenat	Aqueous	7.16
	Methanolic	9.54
	Hydroalcoholic	10.34
<i>Allium stracheyi</i> Baker.	Aqueous	6.38
	Methanolic	7.92
	Hydroalcoholic	9.64

Phytochemical investigation of herbal extracts

Methanolic and hydroalcoholic leaves extract of *Caesulia axillaris* Roxb demonstrated the highest phytochemical constituents. However, aqueous extract showed the less phytochemical constituents. It was found as a rich source of alkaloids, tannins, flavonoids, and phenols.

Table 2. Phytochemical investigation of *Caesulia axillaris* Roxb leaves extracts

Phytochemical	<i>Caesulia axillaris</i> Roxb leaves extracts		
	Aqueous	Methanolic	Hydroalcoholic
Alkaloids	+	+	++
Cardiac Glycosides	—	—	—
Tannins	+	+	++
Saponins	—	+	+
Steroids	—	+	+
Flavonoids	—	+	++
Phenols	+	+	++
Reducing sugars	—	—	—
Proteins	—	—	—

+++; Abundance, ++; Moderate; +; Presence -; Absent

Clerodendrum viscosum Ventenat leaves extract exhibited the rich phytochemicals i.e., alkaloids, steroids, flavonoids and coumarins as well. Aqueous extract exhibited a moderate level of phytochemicals.

Table 3. Phytochemical investigation of *Clerodendrum viscosum* Ventenat leaves extract

Phytochemical	<i>Clerodendrum viscosum</i> Ventenat leaves extract		
	Aqueous	Methanolic	Hydroalcoholic
Alkaloids	+	+	++
Glycosides	+	+	+
Tannins	—	+	+
Saponins	—	+	+
Steroids	+	+	++
Flavonoids	—	+	++
Phenols	+	+	+
Reducing sugars	—	—	—
Proteins	—	—	—
Coumarins	—	—	+

+++; Abundance, ++: Moderate; +: Presence -: Absent

Allium stracheyi Baker. leaves extract showed the presence of rich components i.e., steroids, flavonoids, and phenol. Hydroalcoholic leaves extract was reported for highest phytochemical constituents. Reducing sugar and protein were found absent.

Table 4. Phytochemical investigation of *Allium stracheyi* Baker. leaves extract

Phytochemical	<i>Allium stracheyi</i> Baker. leaves extract		
	Aqueous	Methanolic	Hydroalcoholic
Alkaloids	+	+	+
Tannins	—	+	+
Saponins	+	+	+
Steroids	—	+	++
Flavonoids	+	+	++
Phenols	—	+	+
Reducing sugars	—	—	—
Proteins	—	—	—

+++; Abundance, ++: Moderate; +: Presence -: Absent

Isolation of bioactive compounds

TLC analysis of medicinal plants

In methanolic leaves extract, the Rf value was observed as 0.69 in methanolic leaves extract of *Caesulia axillaris* Roxb. In hydroalcoholic fraction, Rf value was observed as 0.76 in the n-Hexane: ethyl acetate: methanol (70:15:15). However, *Clerodendrum viscosum* Ventenat, hydroalcoholic fraction exhibited the Rf value of 0.81. Moreover, Leaves extract of *Allium stracheyi* Baker. (hydroalcoholic fraction), exhibited the Rf value as 0.84.

Table 5. TLC analysis of medicinal plants

Herbal extract	Extract fraction	Solvent system	Rf Value
Leaves extract of <i>Caesulia axillaris</i> Roxb	Methanolic	n-Hexane: ethyl acetate: methanol (70:15:15)	0.69
	Hydroalcoholic	n-Hexane: ethyl acetate: methanol (70:15:15)	0.76
Leaves extract of <i>Clerodendrum viscosum</i> Ventenat	Methanolic	n-Hexane: ethyl acetate: methanol (70:15:15)	0.71
	Hydroalcoholic	n-Hexane: ethyl acetate: methanol (70:15:15)	0.81
Leaves extract of <i>Allium stracheyi</i> Baker.	Methanolic	n-Hexane: ethyl acetate: methanol (70:15:15)	0.75
	Hydroalcoholic	n-Hexane: ethyl acetate: methanol (70:15:15)	0.84

HPTLC Analysis

In HPTLC analysis, leaves extract of *Caesulia axillaris* Roxb showed the expected compounds as Phenylpropene, and *Clerodendrum viscosum* Ventenat reported as phenolic compounds. Moreover, leaves extract of *Allium stracheyi* Baker. demonstrated as flavonoids and phenol when observed in n-Hexane: ethyl acetate: methanol (70:15:15).

Table 6. HPTLC analysis of medicinal plants

Herbal extract	Solvent system	Rf Value	Expected compounds
Leaves extract of <i>Caesulia axillaris</i> Roxb.	n-Hexane: ethyl acetate: methanol (70:15:15)	0.74	Phenylpropene
	n-Hexane: ethyl acetate: methanol (70:15:15)	0.79	
Leaves extract of <i>Clerodendrum viscosum</i> Ventenat	n-Hexane: ethyl acetate: methanol (70:15:15)	0.73	Phenolic compounds
	n-Hexane: ethyl acetate: methanol (70:15:15)	0.81	
Leaves extract of <i>Allium stracheyi</i> Baker.	n-Hexane: ethyl acetate: methanol (70:15:15)	0.76	Flavonoids & Phenol
	n-Hexane: ethyl acetate: methanol (70:15:15)	0.85	

Determination of Total Phenolic Content (TPC)

TPC was estimated as 68.42 mg and 62.28 mg in the methanolic and hydroalcoholic leaves extract of *Caesulia axillaris* Roxb, respectively. *Clerodendrum viscosum* Ventenat showed the TPC as 57.21 mg and 54.39 mg in methanolic and hydroalcoholic fractions, respectively. Demonstrated the total phenolic content as 64.29 mg and 59.63 mg, respectively. It was observed higher in methanolic extract than hydroalcoholic.

Table 7. Determination of TPC in medicinal plants

Leaves extract	Total Phenolic Content (mg)	
	Methanolic	Hydroalcoholic
<i>Caesulia axillaris</i> Roxb	68.42	62.28
<i>Clerodendrum viscosum</i> Ventenat	57.21	54.39
<i>Allium stracheyi</i> Baker.	34.56	29.23

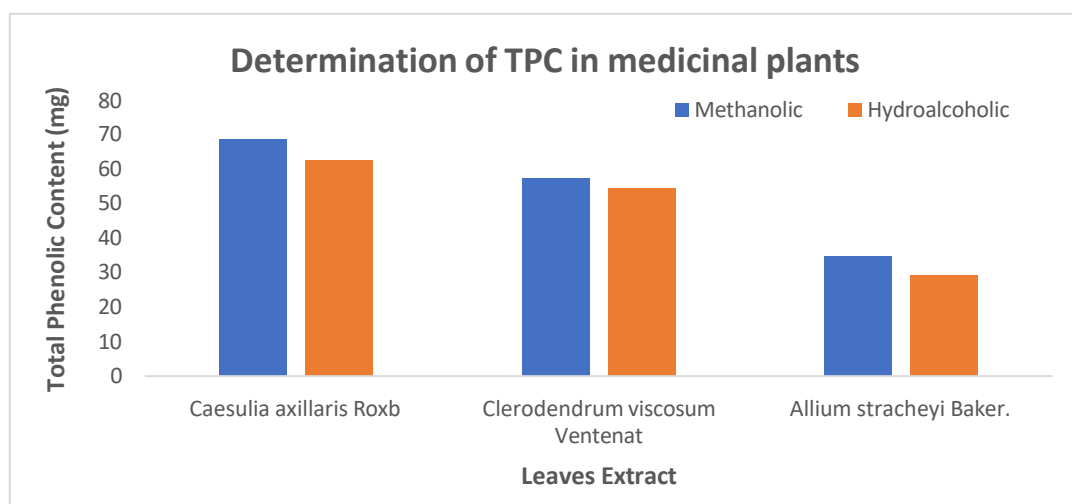


Fig 4. Determination of TPC in medicinal plants

Determination of Total Flavonoids Content (TFC)

TFC was found as 59.42 mg, 104.65 mg and 13.27 mg in methanolic leaves extract of *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat and *Allium stracheyi* Baker. respectively.

Table 8. Determination of TFC in medicinal plants

Leaves extract	Total Flavonoids Content (mg)	
	Methanolic	Hydroalcoholic

<i>Caesulia axillaris</i> Roxb	59.42	42.16
<i>Clerodendrum viscosum</i> Ventenat	104.65	97.31
<i>Allium stracheyi</i> Baker.	13.27	11.87

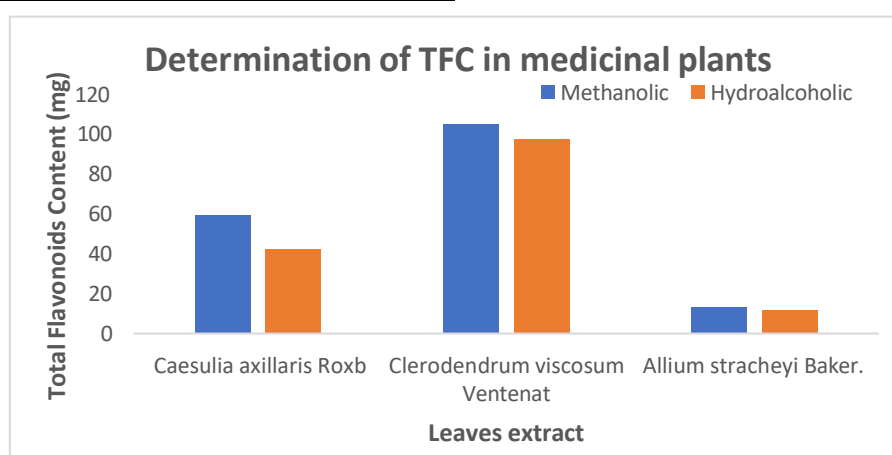


Fig 5. Determination of TFC in medicinal plants

Preliminary phytochemical analysis was performed on the ethyl acetate extract of *Clerodendrum viscosum* Ventenat leaves, and the HPTLC method was used to identify the flavonoid component. The fractions eluted with 90% and 80% chloroform in methanol in column chromatography reacted favorably with the Folin-Ciocalteu reagent. Using HPTLC methods and a mobile phase made up of a chloroform:methanol solvent mixture at a ratio of 11.8:0.2 v/v, the flavonoid from the leaf extract of *Clerodendrum viscosum* Ventenat was discovered. A single flavonoid band with a

peak at R_f 0.25 was seen in the fractions obtained from 90% and 80% chloroform in methanol mixtures [21].

Gallic acid, a polyphenolic chemical, was used as the standard in the measurement of total phenol. Since it is present in almost every plant, it can be used as a standard for measurement. The phenolic content of these organic acids is constant and pure [22]. Since quercetin is a flavonoid that belongs to the flavonol group, it was used as a reference solution in the measurement of total flavonoid levels [23]. The results of this investigation on the phenolic and flavonoid content of the tamarillo peel ethanol

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extract are different from those of previous studies that used different methods and solvents. Different concentrations of flavonoids may be caused by the extraction technique, as well as differences between farmers, growing environments, nutritional levels, and temperatures [24]. According to this study, tamarillo peel ethanol extract has high antioxidant properties. This study is similar to Hawa et al.'s research, which claims that various types have potent antioxidant capacity [25].

CONCLUSION

It concluded that all three plants i.e., *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat and *Allium stracheyi* Baker. taken in the study demonstrated as a rich source of phytochemicals including alkaloids, cardiac glycosides, tannins, saponins, coumarins, steroids and flavonoids in descending order of hydroalcoholic>methanolic>aqueous. However, Total phenolic content and Total Flavonoids content were found higher in methanolic extracts than hydroalcoholic herbal extracts which might be due to better solubility of phytoconstituents.

In further studies, bioactive constituents might be isolated from the *Caesulia axillaris* Roxb, *Clerodendrum viscosum* Ventenat and *Allium stracheyi* Baker. which could be isolated in the management of various diseases.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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