Effect of solvent on the phytochemical screening of Swertia Chirayita plant extract: A comparative study

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Abstract-Swertia Chirayita is one of the widely used herbs for different therapeutic purposes. This paper compares the phytochemical screening of plant extract using two different polarity solvents viz; hydro ethanol and ethyl acetate. Phytochemical screening of the extracts of Swertia Chirayita was performed with spot tests as the standard method. The ethanol extracts showed the presence of saponins, terpenoids/steroids, and flavonoids. The ethyl acetate extract has contents of terpenoids/steroids, and flavonoids. Results showed that different solvents possibly have different phytochemical compounds on extraction.

INTRODUCTION

Medicinal plants have always been used for therapeutic applications, either in the form of traditional preparations or as pure active principles, and they are frequently the only source of medicine for the majority of people.[1] They are a significant source of various phytochemicals such as flavonoids, phenols, saponins, alkaloids, vitamins, minerals, and carbohydrates [2]. The plant-based medicines are common as a source of primary healthcare in many parts of the world because they are rich in phytochemicals and secondary metabolites with medicinal properties. From time to time, thousands of phytochemicals, such as alkaloids, phenolics, flavonoids, saponins, anthocyanins, and terpenoids, have been isolated and identified from numerous plants due to their pharmacological properties.[3]

Swertia Chirayita, commonly known as Chirayita is a traditional Ayurvedic herb that is generally used to treat a variety of ailments like malaria, diabetes, and liver disorders. This herb is mostly distributed in the temperate Himalayas and is an annual, erect plant that has a height of about 0.5 to 1.5 meters. Chirata is a plant with a high potential value that may be helpful in several ailments.[4]

The whole plant is widely used by local people for the treatment of hepatitis, inflammation, and digestive diseases [5]. The wide range of medicinal uses includes the treatment of chronic fever, malaria, anaemia, bronchial asthma, hepatotoxic disorders, liver disorders, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, ulcers, scanty urine, hypertension, melancholia, and certain types of mental disorders, secretion of bile, blood purification, and diabetes. Recently, *S. Chirayita* extracts showed anti-hepatitis B virus (anti-HBV) activities[6]. Traditionally, decoctions of this species are used for anthelmintic, hepatoprotective, hypoglycaemic, antimalarial, antifungal, antibacterial, cardio stimulant, antifatigue, anti-inflammatory, antiaging, antidiarrheal, protectant of the heart and also help in lowering blood pressure and blood sugar.[7] Many herbal preparations contain Swertia Chirayita extract in different concentrations for its antipyretic, hypoglycaemic, antifungal, and antibacterial properties.[8]

The current study was conducted to compare the effect of solvent on the preparation of plant extract with highly bioactive compounds[9,10]. The extract was checked by a phytochemical screen to evaluate the significant presence of dominant compounds. The study would offer the primary platform and worthy information for more studies in the future to apply Swertia Chirayita extract on a larger scale.

MATERIALS AND METHODS

Materials. Fresh Swertia Chirayita plants were collected from the Bairagarh region of Bhopal district,

M P, India. They were washed with water, the leaves and stems dried in the shade and powdered.

Sample Preparation. The two different plant extracts were prepared using hydro ethanol (1:10), and ethyl acetate by the Soxhlet method. Then, the solvent was evaporated using a rotary evaporator, and the crude extracts were kept in the refrigerator. The crude extracts were tested for the presence of alkaloids, steroids, tannins, saponins, flavonoids, and glycosides[11,12]. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals.

METHOD

Qualitative phytochemical analysis

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids are confirmed by the formation of the yellow-coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. The formation of a red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to a test for glycosides.

a) Legal Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. The formation of a 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with a few drops of lead acetate solution. The formation of a yellow colour precipitate indicates the occurrence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with a few drops of conc. Nitric acid. The formation of a yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. The formation of an emerald green colour indicates the presence of diterpenes.

Quantitative studies of phytoconstituents

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method[13]. 10 mg Gallic acid was dissolved in 10 ml methanol, and various aliquots of 10- 50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoid content

The determination of total flavonoid content was based on the aluminium chloride method [14]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

RESULTS AND DISCUSSIONS

Phytochemical screening

Qualitative phytochemical tests of Ethyl acetate extract and Hydro ethanol extract of the Swertia Chirayita plant prepared by the Soxhlet extraction method were performed separately. The results of various tests are summarized in Table 1. Phytochemical screening of Swertia Chirayita plant

extract with other solvents like methanol[15], acetone[16], and ethanol has been done by many other researchers as well. Flavonoids, phenols and carbohydrates were commonly found in both the extracts prepared.

Table 1: Result of phytochemical screening of Soxhlet extraction

S. No.	Constituents	Ethyl acetate extract	Hydro ethanol Extract
1.	Alkaloids Hager's Test:	 -ve	 +ve
2.	Glycosides Legal's Test:	 +ve	 -ve
3.	Flavonoids Lead acetate Test: Alkaline test:	 +ve +ve	 +ve +ve
4.	Diterpenes Copper acetate Test:	 -ve	 +ve
5.	Phenol Ferric Chloride Test:	 +ve	 +ve
6.	Proteins Xanthoproteic Test:	 -ve	 +ve
7.	Carbohydrate Fehling's Test:	 +ve	 +ve
8.	Saponins Froth Test:	 -ve	 +ve
9.	Tannins Gelatin test:	 -ve	 -ve

+ve = present, -ve =negative

RESULT OF ESTIMATION OF TOTAL PHENOL AND FLAVONOID CONTENT OF EXTRACT

Estimation of total phenolic content (TPC)

Total phenolic compounds (TPC) were expressed as mg/100mg of the gallic acid equivalent of the dry extract sample using the equation obtained from the calibration curve: y = 0.014x + 0.004, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Dutta et al[17]. reported that aqueous extract had the highest level of phenolic content (221 ± 12 mg GAE/100g) while ethanol 100% with TPC of (20 ± 2 mg GAE/100g) and Khanal et al[15]. reported that methanolic extract with 67.49±0.50 mg GAE/g in Swertia Chirayita.

Calibration Curve of Gallic acid

Table 2: Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.145
2	20	0.302
3	30	0.442
4	40	0.569
5	50	0.721

(n=3, Mean ± SD)

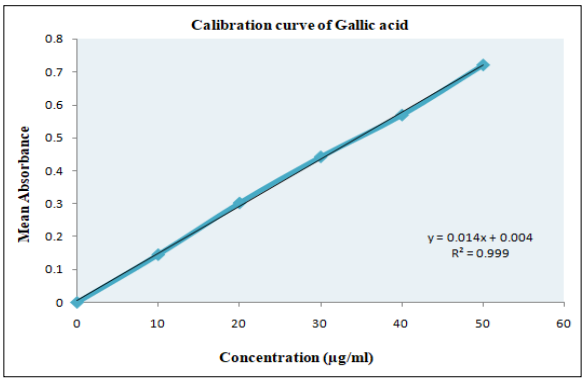


Figure 1: Graph of calibration curve of Gallic acid

Estimation of total flavonoid content (TFC)

Total flavonoid content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.021x + 0.008, R^2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. Chen et al[18]. estimated the TFC to be 4.98 ± 0.40 mg rutin equivalents/g in ethanol extract, Tripathi et al[19]. reported 10.6 µg equivalents of quercetin in 50 µg of aqueous extract and Khanal et al[15] reported 26.16 ±0.25mg QE/g in Swertia Chirayita.

Calibration curve of Quercetin

Table 3: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.123
2	10	0.225
3	15	0.337
4	20	0.44
5	25	0.541

(n=3, Mean ± SD)

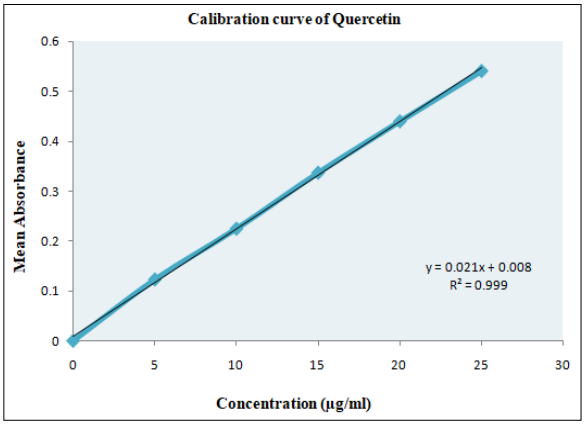
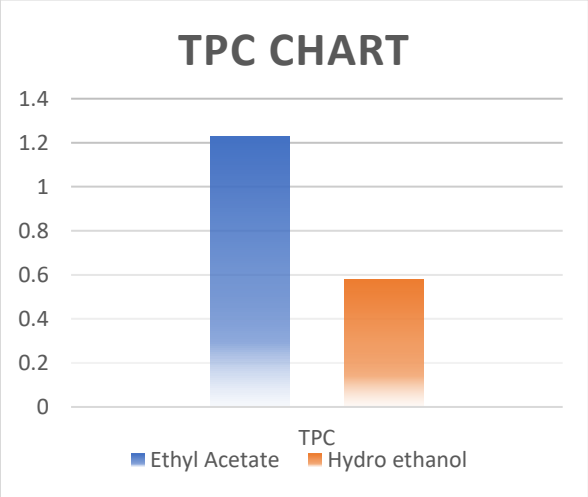


Figure 2: Graph of calibration curve of Quercetin

Table 4: Estimation of total phenol and flavonoid content of Soxhlet extraction

S. No.	Extract	Total phenol content	Total flavonoids Content
1.	Ethyl acetate	1.23 mg/ 100 mg	6.41mg/ 100 mg
2.	Hydro ethanol	0.58mg/ 100 mg	3.03mg/ 100 mg



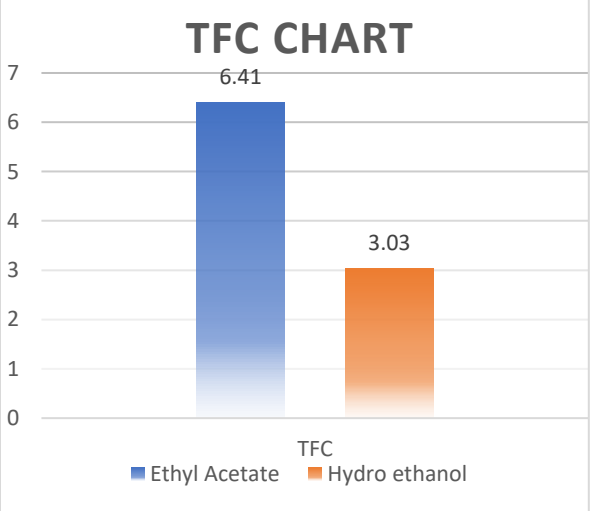


Figure 3: TPC and TFC chart

CONCLUSION

The results of the research can be concluded that the hydro ethanol extract of Swertia Chirayita contains phytochemical compounds like alkaloids, saponins, proteins, terpenoids, and flavonoids. The ethyl acetate extracts of the plant show the presence of glycosides, terpenoids and flavonoids. The ethanolic extract has more complete phytochemical compounds than ethyl acetate extracts. Moreover, the total phenolic contents and total flavonoid contents were found to be richer in ethyl acetate extract than the ethanolic extract of the Swertia Chirayita plant.

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