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RESEARCH ARTICLE

Phytochemical Screening and Analysis of Antioxidant Activity of *Crateva unilocularis* Buch.-Ham. Leaf

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ABSTRACT

Crateva unilocularis (Capparaceae) is commonly known as siplegan in Nepal. It is a widely used medicinal plant distributed throughout Nepal at an altitude of 100 - 1800 meters. In the present study preliminary phytochemical screening and in-vitro antioxidant property of hexane, chloroform and methanol soluble fraction of leaf extract was investigated. Phytochemical screening of methanolic fraction showed the presence of carbohydrates, saponins, flavonoids, phytosterols, fixed oils, fats and phenols. The hexane fraction showed the presence of fewer active ingredients when compared with the other fractions. Carbohydrates, fixed fats, oils and phenols are present only in methanol soluble fraction. Alkaloids, resins, flavonoids, protein and amino acids are absent in methanol soluble fraction. The methanolic fraction showed the presence of more phytochemicals when compared to the hexane and chloroform soluble fractions. Among all three fractions, hexane soluble fraction had the least number of phytochemicals. The reason behind this was due to the ability of methanol to extract both polar and non-polar phytochemicals whereas hexane can only extract non polar phytochemicals. The antioxidant sensitivity test showed that the hexane, chloroform and methanolic fractions showed antioxidant activity in concentration dependent manner. Methanolic fraction showed up to 92% inhibition of DPPH free radical at 517 nm at high concentration of 1 mg/ml. The presence of strong antioxidant activity of chloroform and methanolic fractions of *C. unilocularis* can be a great scope for further studies on this plant regarding its antioxidant potentials.

KEYWORDS

Crateva unilocularis, Antioxidant activity, DPPH assay, Phytochemical screening

INTRODUCTION

The medicinal value of plants lies in the specific chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. It is necessary to determine the nature of chemical constituents of the plant to determine its biological activity.

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The phytochemical research based on ethno pharmacological information is an effective approach in the discovery of new anti-infective agents from higher plants.[1]

Crateva unilocularis (Capparaceae) is commonly known as siplegan in Nepal. It is a widely used medicinal plant distributed throughout Nepal at an altitude of 100-1800 meters. Various traditional uses of C. unilocularis are reported. The bark has laxative, stomachic and antiperiodic properties and the juice of young leaves is used traditionally for the treatment of helminth infestations.[2] Leaves

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are used in rheumatism. The juice of bark is taken to treat fever and urinary complaints. A paste of the fruit is applied to treat smallpox. Roots and bark promote appetite and increase biliary secretion.[3] Methanolic extracts of leaves of *C. unilocularis* shows good in vitro antibacterial activity against Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli whereas the methanolic extracts of the bark shows activity against S. aureus and E. coli only.[4]

Figure 1: *C. unilocularis* Leaves with flowering parts

Reactive oxygen species (ROS) are implicated in a wide range of human diseases including atherosclerosis, stroke and diabetic retinopathy. When an imbalance between generated ROS and available antioxidants occurs, oxidative damage will spread *via* free radical generation in many cellular materials (*e.g.*, DNA, lipids, and proteins). For this reason, the chemical natures and quantities of antioxidants in foods and medicinal plants have attracted much interest in recent years.[5]

There are a number of clinical studies suggesting that the antioxidants in various parts of medicinal plants, fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers.

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The molecule, 2, 2-Diphenyil-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule do not dimerise, as would be the case with most other free radicals. The delocalization gives rise to the deep violet colour, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives reduced form with the loss of the violet colour. The pale colour may appear at the end due to still presence of picryl residue.[6, 7]

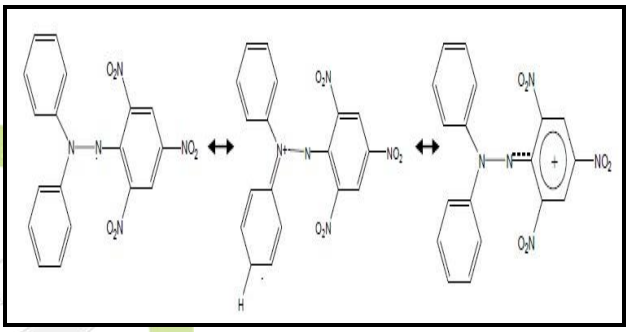

Figure 2: Structure of stable DPPH free radical

MATERIALS AND METHODS

Plant Material

Leaves of *C. unilocularis* were collected from Lekhnath Municipality-12, Kaski, Nepal during July, 2011. Only mature and healthy leaves were collected and any dirt, dust or insects on leaf surfaces were removed. The herbarium specimen of the plant was identified by National Herbarium and Plant Laboratory, Godawari, Lalitpur Nepal as *Crateva Unilocularis.*

Extraction was done as per the chart given.

Phytochemical Screening[8]

Following test were carried out for the phytochemical screening of all three extracts:

Alkaloids Test

Each of the three extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were separately treated with Mayer's, Wagner's and Hager's Reagent to determine the presence of alkaloids.

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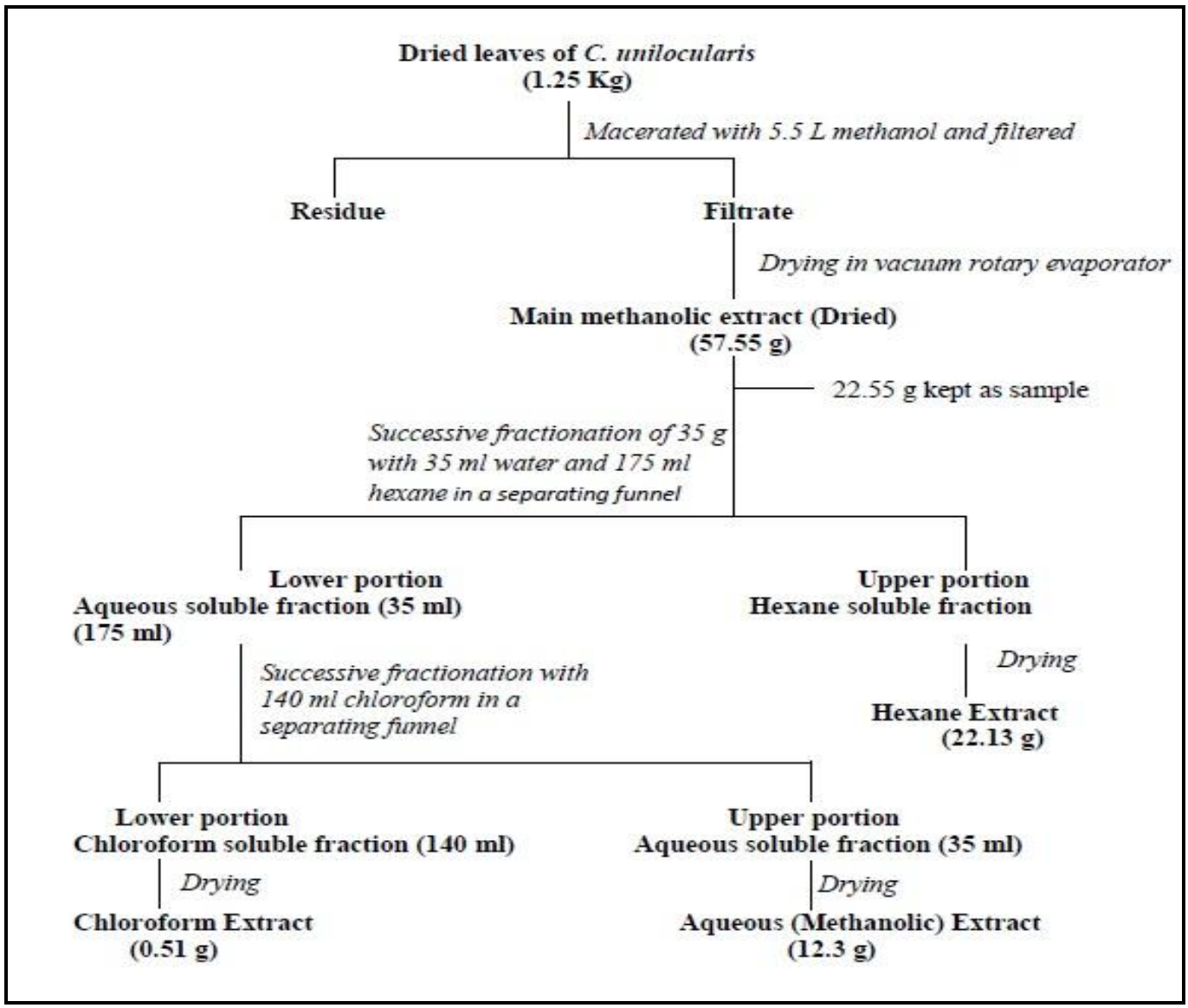

Figure 3: Extraction process

Carbohydrates Test

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were separately treated with Molish's Reagent to determine the presence of carbohydrates.

Saponins Test

Extracts were shaken vigorously with 20 ml of water in a test tube for few minutes, a process known as Foam test

Phytosterols Test

It was done by Salkowski's test.

In Salkowski's test method, extracts were treated with chloroform and filtered. The filtrates were then treated with few drops of concentrated sulfuric acid and allowed to stand and the result was observed.

Fixed Oils and Fats Test

It was done by filter paper press test in which extracts were pressed in filter paper and results were observed.

Resins Test

It was done by acetone water test in which extracts were treated with 5 ml acetone; equal

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volume of water was added and shaken and results were observed.

Phenols Test

It was done by treating the extracts with 5 ml of FeCl3 solution.

Tannins Test

It was done by gelatin test method in which extracts were treated with 5 ml of 1% Gelatin solution containing NaCl and results were observed.

Flavonoids Test

It was done by alkaline reagent test.

In Alkaline Reagent test, extracts were treated with 5 ml of sodium hydroxide and observed.

Proteins and Amino Acids Test

It was done by Xanthoproteic test.

In Xanthoproteic test, extracts were treated with 2 drops of concentrated HNO3 and observed.

Determination of Antioxidant Activity

***DPPH Radical-Scavenging Activity Test*[9]**

To determine the antioxidant activity of the various fractions of the extract, DPPH radical-scavenging activity test was adopted. Stock solutions of concentration 1.0 mg/ml of each fraction (Hexane, Chloroform and Methanol) as well as of the standard (Ascorbic acid) were prepared using methanol as solvent. From the stock solution of each fraction and standard solution, serial dilution with methanol was done to prepare test solutions of concentration 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml. These solutions of concentration from 1.0 mg/ml to 0.001 mg/ml were used to determine the antioxidant activity. DPPH solution of concentration 60 μM was freshly prepared and 2 ml of it was mixed with 2 ml of each test solutions of each fraction and the standard (Ascorbic acid) solution. The resulting 4 ml solution was allowed to stand for 30 minutes. After 30 minutes, the absorbance of each reaction mixture was measured in UV Spectrophotometer at 517 nm. The absorbance reading was then recorded for each solutions and the percentage of free radical scavenging activity was calculated from the given formula. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The capability to scavenge the DPPH free radical was calculated using the following equation:

% of radical scavenging activity = (Abs control – Abs sample)/(Abs control) X 100%

Where,

Abs control = Absorbance of DPPH solution

Abs sample = Absorbance of extracts and ascorbic acid solutions

RESULTS AND DISCUSSION

Phytochemical investigation of methanolic, hexane and chloroform fractions of *C. unilocularis* leaves has revealed differences in their phytoconstituents. Extracts obtained using different solvents showed differences in their constituents as well.

Phytochemical screening of methanolic fraction showed the presence of carbohydrates, saponins, flavonoids, phytosterols, fixed oils, fats and phenols. The phenolic compound has several functions such as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors and synergists.[10] the hexane fraction showed presence of fewer active ingredients when compared with the other fractions. Carbohydrates, fixed fats, oils and phenols were present only in methanol soluble fraction. Alkaloids, resins, flavonoids, protein and amino acids were absent in methanol soluble fraction. The methanolic fraction showed the presence of more phytochemicals when compared to the hexane and chloroform soluble fractions. Among all three fractions, hexane soluble fraction had the least number of phytochemicals. The reason behind this is due to the ability of methanol to extract both polar and non-polar phytochemicals whereas hexane can only extract non polar phytochemicals.

Phytochemical Screening of Hexane Soluble Fraction

Table: 1 Results of Phytochemical Screening of Hexane Soluble Fraction

S. N.	Phytochemical Test	Reagents used (test performed)	Interference	Result
1.	Alkaloid test	Mayer's Reagent	Appearance of yellow cream ppt.	Negative
		Wagner's Reagent	Formation of brown/reddish brown precipitate	Positive
		Hager's Reagent	Formation of yellow colour ppt.	Negative
2.	Carbohydrate test	Molish's Reagent	Formation of violet ring	Negative
3.	Saponin test	Foam Test	No production of foam	Negative
4.	Phytosterol test	Salkowski's Test	No golden brown colour obtained	Negative
6.	Fixed oils and fats test	Filter Paper press Test	No oily stain was obtained	Negative
7.	Resin test	Acetone Water Test	Appearance of Turbidity	Positive
8.	Phenol test	Ferric Chloride Test	Appearance of bluish black ppt.	Negative
9.	Tannin test	Gelatin Test	No formation of white ppt.	Negative
11.	Flavonoids test	Alkaline Reagent Test	No intense yellow colour obtained	Negative
12.	Proteins and amino acids test	Xanthoproteic Test	No formation of yellow colour	Negative

Phytochemical Screening of Chloroform Soluble Fraction

Table: 2 Results of Phytochemical Screening of Chloroform Soluble Fraction

S. N.	Phytochemical Test	Reagents used (test performed)	Interference	Result
1.	Alkaloid test	Mayer's Reagent	Appearance of yellow cream ppt.	Negative
		Wagner's Reagent	Formation of brown/reddish brown precipitate	Negative
		Hager's Reagent	Formation of yellow colour ppt.	Negative
2.	Carbohydrate test	Molish's Reagent	Formation of violet ring	Negative
3.	Saponin test	Foam Test	Produce foam that lasts for more than 10 minutes	Positive
4.	Phytosterol test	Salkowski's Test	Golden brown colour obtained	Positive
5.	Fixed oils and Fats test	Filter Paper press Test	No oily stain was obtained	Negative
6.	Resin test	Acetone Water Test	Appearance of Turbidity	Positive
7.	Phenol test	Ferric Chloride Test	No appearance of bluish black ppt.	Negative
8.	Tannin test	Gelatin Test	No formation of white ppt.	Negative
9.	Flavonoids test	Alkaline Reagent Test	No intense yellow colour obtained	Negative
10.	Proteins and amino acids test	Xanthoproteic Test	No formation of yellow colour	Negative

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Phytochemical Screening

Phytochemical Screening of Methanol Soluble Fraction

Table: 3 Results of Phytochemical Screening of Methanolic Soluble Fraction

S. N.	Phytochemical Test	Reagents used (test performed)	Interference	Result
1.	Alkaloid test	Mayer's Reagent	Appearance of yellow cream ppt.	Negative
		Wagner's Reagent	Formation of brown/reddish brown precipitate	Negative
		Hager's Reagent	Formation of yellow colour ppt.	Negative
2.	Carbohydrate test	Molish's Reagent	Formation of violet ring	Positive
3.	Saponin test	Foam Test	Produce foam that lasts for more than 10 minutes	Positive
4.	Phytosterol test	Salkowski's Test	Golden brown colour obtained	Positive
6.	Fixed oils and fats test	Filter Paper press Test	Oily stain was obtained	Positive
7.	Resin test	Acetone Water Test	No appearance of Turbidity	Negative
8.	Phenol test	Ferric Chloride Test	Appearance of bluish black ppt.	Positive
9.	Tannin test	Gelatin Test	No formation of white ppt.	Negative
11.	Flavonoids test	Alkaline Reagent Test	No intense yellow colour obtained	Negative
12.	Proteins and amino acids test	Xanthoproteic Test	No formation of yellow colour	Negative

*positive→ Presence
*negative→ Absence

Antioxidant Sensitivity Test

Absorbance of each solution were measured and recorded as follows

Table 4: UV absorbance of extracts and standard at 517 nm

Concentration (mg/ml)	Abs* hexane fraction	Abs* Chloroform fraction	Abs* Methanolic fraction	Abs* Ascorbic acid
0.001	0.326666667	0.319666667	0.320333333	0.278666667
0.01	0.322333333	0.301666667	0.312666667	0.016333333
0.1	0.298	0.215666667	0.232666667	0.013666667
1	0.251	0.115666667	0.024	0.013

*Absorbance

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Table 5: Percentage Inhibition of DPPH free radical by extracts/standard at 517 nm

Concentration (mg/ml)	% inhibition by hexane fraction	% inhibition by chloroform fraction	% inhibition by methanolic fraction	% inhibition by Ascorbic acid
0.001	2.777777778	4.861111111	4.662698413	17.06349206
0.01	4.067460317	10.21825397	6.944444444	95.13888889
0.1	11.30952381	35.81349206	30.75396825	95.93253968
1	25.29761905	65.57539683	92.85714286	96.13095238

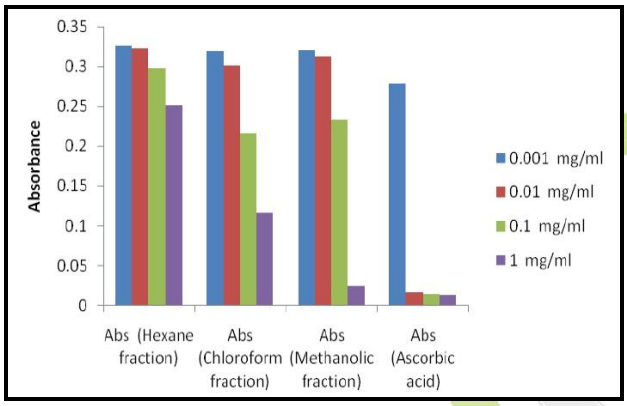

Figure 3: UV absorbance of extracts and standard at 517 nm

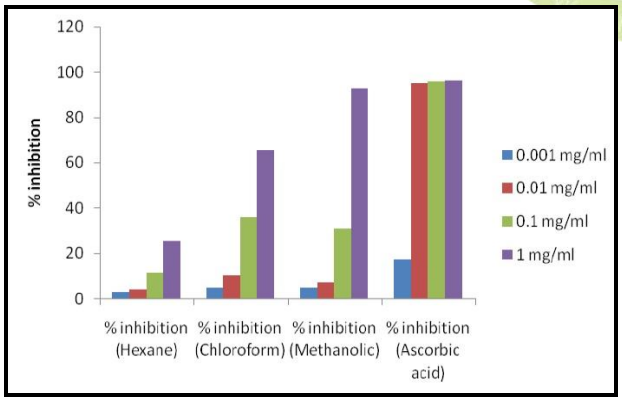

Figure 4: Percentage Inhibition of DPPH free radical by extracts and ascorbic acid at 517 nm

The DPPH radical scavenging test is a widely used model system to investigate the scavenging activities of several natural compounds including phenolic compounds, flavonoids or crude mixtures of plants.[11] The antioxidant sensitivity test showed that the hexane, chloroform and methanolic fractions showed antioxidant activity in concentration dependent manner. The methanolic fraction showed up to 92% inhibition of DPPH free radical at 517 nm at high concentration of 1 mg/ml. The hexane soluble fraction showed least antioxidant efficacy due to the presence of non-polar constituents. At the concentrations of 0.01 mg/ml, 0.1 mg/ml and 1mg/ml, ascorbic acid shows similar activity of about 95% inhibition of DPPH free radicals. This might be due to the complete reaction of ascorbic acid with the DPPH free radicals present in the solutions and there are no free radicals remaining in the solution to react with ascorbic acid. That is, all DPPH free radicals are consumed at 0.01 mg/ml solution of ascorbic acid.

CONCLUSION

C. unilocularis is an important medicinal plant in the traditional Ayurvedic system of medicine and is widely used traditionally for the remedy of various ailments and diseases. This study has discovered the various phytochemicals, including alkaloids, phenols and saponins, present in the leaves of *C. unilocularis.* The presence of strong antioxidant activity of chloroform and methanolic fractions of *C. unilocularis* can be a great scope for further studies on this plant regarding its antioxidant potentials. Therefore further research on this plant is necessary to flourish its medicinal properties and therapeutic uses.

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