

**PHARMACOGNOSTICAL EVALUATION AND PHYTOCHEMICAL
PROFILING OF IMPORTANT PLANT SPECIES *Zizhyphus Oenoplia L.***

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ABSTRACT

The present study was aimed to carry out the pharmacognostic and phytochemical study on the three important medicinal plants available in this area *Ziziphus oenoplia L.* belongs to the family Rhamnaceae. The pharmacognostic study includes the parameters like morphological observations including root length, shoot length, no. of lateral roots, internodes length and leaf surface area was observed and tabulated. The anatomical parameters *viz.*, transverse section of the study plant was observed with the treatment of various chemicals and solvents. Similarly, the powder studies were also carried out. Finally, the qualitative phytochemical studies were carried out to find the presence of secondary metabolites in the extract. The extracts exhibited a variety of important phytochemicals *viz.*, Alkaloids, Tannins, Saponins, oils, Steroids and Terpenoids. Further the anatomical study and powder showed the characteristic colour change and physical changes which determined the genuinity of the species.

Keywords: Phytochemicals, pharmacognosy, Alkaloids, *Zizhyphus oenoplia*

PHARMACOGNOSTICAL EVALUATION AND PHYTOCHEMICAL PROFILING OF IMPORTANT PLANT SPECIES *ZIZHYPHUS OENOPLIA* L.

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ABSTRACT

The present paper deals with determine the quality parameters and also to authenticate the species free from adulteration, the present study was carried out on pharmacognostically and phytochemically on three important medicinal plants available in this area *Ziziphus oenoplia* L. belongs to family Rhamnaceae. The pharmacognostical work includes the parameters such as morphological observations including root length, shoot length, no. of lateral roots, internodes length and leaf surface area were observed and tabulated. The anatomical parameters *viz.*, transverse section of the study plant was observed with the treatment of various chemicals and solvents. Similarly the powder studies were also carried out. Finally the qualitative phytochemical studies were carried out to know the presence some basic secondary metabolites in the extract. The extracts exhibited the presence of important phytochemicals *viz.*, Alkaloids, Tannins, Saponins, Oils, Steroids and Terpenoids. Further the anatomical study and powder showed the characteristic colour change and physical changes determines the genuinity of the species.

Keywords: Phytochemicals, pharmacognosy, Alkaloids, *Zizhyphus oenoplia*

INTRODUCTION

Medicinal plants are used by 80% of the world population for their basic health needs. India is the birth place of indigenous medicines such as Siddha, Ayurveda and Unani system. All those systems rely on medicinal plants. The medicinal plants a very importance place in day to day life of human beings through on the world from immemorial. The plants have become knowing or unknowingly become parts of the home remedial system. Hence the relationship with the plants was extended key and medicinal aspects. Traditional system of medicines was prepared from a single combination of more than one plant. The

efficacy of the drug depends upon the on quality of plant species, parts and biological property of medicinal plants which in turn depends upon the occurring as primary and secondary metabolites (Phuspa,2009). Intern, it is necessary to have detailed knowledge on medicinal plants used for preparedly a drug in every aspect of the medicinal plants are rich source of various chemical compounds present. Medicinal plants are still major parts of traditional medicinal systems in developing countries many infection disease are known to be treated with herbal remedies throughout the history of mankind. Even today plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Sukanya *et al.*, 2009). Medicinal plants which from backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plant as potential source of new compounds of therapeutic values and as source of new compounds in drug development (Buduru Gowthami *et al.*, 2012)

The branch of pharmacology that deals with drugs in the form of crude or natural state of medicinal herbs or other plants for their original features is called pharmacognosy. It is also the study of medicines derived from natural sources, mainly from plants and deals with standardization, authentication and study of natural drugs.

Most of the research in pharmacognosy has been done in identifying controversial species of plants, authentication of commonly used traditional medicinal plants through organoleptic, anatomical, morphological, phytochemical and some physicochemical analysis. Recently the importance of pharmacognosy in taxonomic identification, pharmacognostic study includes parameters which help in identifying adulteration in dry powder and extract form also. The importance of the study in plants is gained because when the plant was dried and made into powder form, it loses its morphological identity and easily prone to adulteration. The study ensures plants identity, lays down standardization parameters which will help and prevents adulterations in dry powder form also. This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. Pharmacognostic studies ensure plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. The pharmacognostic standardization parameters which are generally done are described below (Sumitra, 2014). The pharmacogonostic characters will be very specific to every

species, locality and environmental condition. The organoleptic study on the plants which will give rise to reaction of live cells on various chemical treatments. Hence the study mainly gains its importance to know complete characters of the species and details of anatomical and powder level ingredients.

To determine the quality parameters and also to authenticate the species free from adulteration, the present study was carried out on pharmacognostically and phytochemically on three important medicinal plants available in this area *Ziziphus oenoplia* L. belongs to family Rhamnaceae respectively. Indeed these three plants belong to diverse families but inhabit the similar soil and climatic conditions. The plants were very used for the various purposes in the villages. Commonly the indigenous people use these plants or allow the plants to grow as biofencing. The plants do not need regular water supply and survive any type of soil. The artificial regeneration was poorly established but naturally the plants found. The study plants are widely distributed in this area. The pharmacognostical work includes the parameters such as morphological observations including root length, shoot length, no. of lateral roots, internodes length and leaf surface area were observed and tabulated. The anatomical parameters *viz.*, transverse section of the study plant was observed with the treatment of various chemicals and solvents. Similarly the powder studies were also carried out. Finally the qualitative phytochemical studies were carried out to know the presence of some basic secondary metabolites in the extract.

MATERIALS AND METHODS

Collection of plant materials:

For the present study the plant species, *Ziziphus oenoplia* belong to family *Rhamnaceae* respectively were collected from nearby areas of Pollachi and Gobichettipalayam and preserved accordingly as the study to be carried out. The plants were collected and shade dried and finely powdered for the powder studies. The fresh plant specimens were stored in a proper fixative like FAA till the sections to be made.

Classification:

Description

The plant belongs to the Rhamnaceae family (Ber family). Common name is jackal jujube. It is a very thorny straggling shrub with rusty-velvety young branches with paired thorns. Scandent shrubs, thorns many, recurved, branchlets densely silky hairy. Leaves 4-6.5 x 2-3 cm, ovate, apex acuminate, base oblique, 3

or 4 ribbed, densely fulvous hairy, sessile or shortly petioled. Flowers 3 mm across, shortly pedicelled, 6-20 in axillary clusters; sepals triangular, hispid outside; petals clawed, concave, greenish yellow; disk flat, glabrous. Drupe 6 x 6 mm, globose, black.

Preparation of powder

The clean and healthy plants were collected. The materials were shade dried and powdered using pulverizer. The finely powdered plant materials were stored properly under proper condition.

Preparation of extract:

The two study plants material was collected separately and dried under shade. The well dried plant material was powdered using pulverizer. The finely powdered plant parts were used to take hot extract by using soxhlet apparatus.

Organoleptic studies:

Microscopic and macroscopic observations of the study plants were carried out like according to Saha *et al.*, 2011.

Microscopic analysis:

Free hand transverse sections leaf, stem, internode, root, petiole were cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol. Kunle *et al.*, 2012.

Determination of Foaming index

Some plant materials when shaken with water cause persistent foam which may be attributed to the presence of saponins in that material. The foaming ability of an aqueous solution of plant materials and their extracts is measured in terms of foaming index WHO guidelines.

An accurate quantity of about 1g of the coarse plant material was weighed and transferred into an Erlenmeyer flask containing 100ml of boiling water. The flask was boiled at moderate heat for 30min. The solution was cooled and filtered into a 100ml volumetric flask and sufficient distilled water was added to dilute to volume. The solution was poured into ten stoppered test tubes in successive portions of 1ml, 2ml, etc. upto 10ml, and the volume of the liquid in each tube was adjusted with water upto 10ml. The tubes were then stoppered and shaken in a length wise motion for 15sec (two shakes/sec) and allowed to stand for 15min. The height of foam was measured. If the height of the foam in every tube was less

than 1cm the foaming index was less than 100. If a height of foam of 1cm was measured in any test tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If the height of the foam was more than 1cm in every tube, the foaming index was over 1000. In this case, the determination was repeated using a new series dilution of the decoction in order to obtain a result. The foaming index was calculated by using the following formula $1000/A$ where A was the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed

Preliminary phytochemical analysis

The obtained extract was screened for the presence of phytochemical such as alkaloids, tannins, flavonoids, saponins, glycosides, saponins, terpenoids, phenol and oil.

a) Detection of alkaloids-Mayer's test (Sofowara *et al.*, 1993)

The mercuric chloride (1.36g) was dissolved in 60 ml of distilled water 5g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent was added. Formation of yellowish buff colored precipitate confirmed the presence of alkaloids.

b) Detection of Flavonoids-H₂SO₄ test (Harborne *et al.*, 1973)

0.5mg of plants was treated with few drops of H₂SO₄. Formation of orange colour indicates that the presence of flavonoids.

c) Detection of Glycosides- Kellar-killani test (Gokhale *et al.*, 2008)

0.5mg of plant extract was shaken with 1 ml of glacial acetic acid. A drop of ferric chloride and a drop of concentrated sulfuric acid were added. Green blue colour to upper layer and reddish brown colour at the junction of two layers indicates the presence of cardiac glycosides.

d) Detection of tannins-FeCl₃ test (Mace and Gorbach, 1963; Ciulci, 1994)

0.5mg of powder sample of each plant is boiled in 20 ml of distilled water in a test tube and filtered 0.1% FeCl₃ is added to the filtered samples and observed for brownish green or a blue black coloration which shows the presence of tannins.

e) Detection of saponins - Vigorous shaking test (Brain and Turner, 1975)

The extract (50 mg) is diluted with distilled water and made up 20 ml, the suspension is shaken in a graduated cylinder for 15 min. A two centimetre layer of foam indicates the presence of saponins.

f) Detection of Terpenoids - Salkowski s Test (Salkowski and Balish, 1987)

0.5mg of the extract of the leaves, flower and seeds was mixed with two ml of chloroform and H₂SO₄ (3 ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates that the presence of Terpenoids

g) Detection of phenol-Ferric chloride test (Harborne, 1973)

10 mg extract were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

h) Detection of steroids (Ciulci, 1994)

Two ml of acetic anhydride was added to five mg of the extracts, with two ml of H₂SO₄. The colour was changed from violet to blue or green in some samples indicates that the presence of steroids.

Table: 1 Showing the quantitative on morphological feature

S.No	Plants name	Stem Length (cm)	Leaf rachis Length (cm)	Single leaf Length (cm)	Petiole Length (cm)	Thorn Length (cm)
2	<i>Ziziphus oenoplia</i>	2	15	4.5	0.3	0.3

Table: 2. Showing the organoleptic characters of study species

S.No	Plant	Part	Colour	Odour	Taste	Texture
1	<i>Ziziphus oenoplia</i>	Stem	Brown	Characteristic	Bitter	Fine
		Leaf	Dark green	Characteristic	Bitter	Fine

Table:3 Showing the results of foaming test

S.No	Plant	Part	Foaming index (1cm)
3	<i>Ziziphus oenoplia</i>	Stem	<1
		Leaf	<1

<1 indicates the occurrence of persistent foam ability of the extract is below 1 cm

**PHARMACOGNOSTICAL, PHYTOCHEMICAL AND
ANATOMICAL EVALUTION OF *CRATEVA RELIGIOSA* G.FORST**

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Abstract

The pharmacognostical, Phytochemical and anatomical characterisation of a medicinal plant is considered to be most important parameter to evaluate and characterise the plant species before making a herbal drug. The present study was carried out on important medicinal plant *Crateva religiosa* G. Forst of Capparidaceae family was made to understand the organoleptic, phytochemical and pharmacognostical characters clearly shows the characteristic features of the plant powders and reactions with various chemical and colour change in appearance was noted to understand the foreign substance presence and absence microscopically. The important phytochemicals such as Alkaloids, Steroids, Terpenoids Flavonoids, Coumarins, Saponins, Glycosides, Phenols and Tannins were found to be present in methanolic extract. The present study clearly shows promising nature of medicinal plant with all important parameters for an herbal drug manufacturing industry.

Keywords: Phytochemicals, pharmacognosy, alkaloids, flavonoids

**IN VITRO ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACTS
OF *HENCKELIA INCANA* (VAHL) SPERNG.**

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ABSTRACT

The study focuses on the antioxidant capabilities of an endemic herb *Henckelia incana* (Vahl) Spreng (syn : *Didymocarpus tomentosa* Wight). This is an unexplored plant species belongs to the family Gesneriaceae, collected from Gopalswami hills, Western Ghats of Tamil Nadu. The leaves of *H. incana* were dried, powdered and methanol extract was extracted using Soxhlet apparatus. Antioxidant activities of the plant extracts was determined by *in vitro* methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Total Antioxidant assay by phosphor molybednum and Reducing power assay. All assays were carried out by the methanol extracts of sample dissolved in DMSO. Ascorbic acid was used as standard. DPPH scavenging of leaf extract was significant and the IC₅₀ value was calculated as 43.10±0.073µg/ml. Dose dependent Reducing power and total antioxidant capacity was observed significantly. This study reveals that *H. incana* extracts have a high antioxidant capacity and may be useful for nutritional and therapeutic purposes.

Keywords: *Henckelia incana*, DPPH, Free radicals, Total Antioxidant activity and reducing power assay

INTRODUCTION

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety.

Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions. They may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Tevfik and Kadir 2008). There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid (Velioglu *et al.*, 1998).

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Marja *et al.*, 1999). As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity (Chew *et al.*, 2012).

Hencalia incana (Vahl) Spreng, (Syn. *Didymocarpus tomentosa* Wight) is a endemic herb, (Prameela *et al.*, 2012) belonging to the family Gesneriaceae, is a common herb found mostly on wet rocks in grass lands, slopping ground, river banks. This plant is widespread in the peninsular India, South India, Srilanka over Sumatra, Philippines. The plant is attractive with bluish- purple flowers in corymbs. It is an ethno- medicinal species, leaves are used in folk medicine for treating skin allergy (Prameela *et al.*, 2012) cure ear pain and it's juice is taken orally to treat fever and as tonic.

Gesneriaceae is a pantropical family, with ca. 150 genera and 3000 species (growing on rocks). The family is commonly divided in two subfamilies, Gesnerioideae (tropical America) and Cyrtandroideae (Old World, Australia, and some Pacific Islands). It is constituted of herbs, lianas, or shrubs, frequently with ornamental potential, due to the beauty of their flowers. Some Species have been used in traditional medicine, mainly against fever, cough, colds, snakebite, pains, and infectious and inflammatory diseases. Although Gesneriaceae are a large family, only few species were chemically investigated, and this took place mainly in the last decade. Many compounds have been reported in Gesneriaceae species, including flavonoids, terpenes and steroids, phenolic glucosides, simple phenolics, quinones, lignans, xanthenes and compounds with unusual skeletons. Several species had been used in folk medicine, and some constituents have

shown biological activities, such as antimicrobial, anti-inflammatory, antioxidant, and antitumor properties (Maria and Maria 2012).

A few species of Gesneriaceae have been used by the indigenous people of tropical Asia and America. Species *D. pedicellata* popularly known as shilapushpa is considered as a valuable medicinal plant in traditional Indian medicine. The plant extract is used for its diuretic properties to maintain a healthy urinary tract and for the treatment of kidney stones. The extracts of the aerial parts of *D. pedicellata* has exhibited potent antioxidant, nephro-protective and antitumor promoting activities (Prameela *et al.*, 2015). Oxidative stress has been postulated in many conditions, including atherosclerosis, inflammatory condition, certain cancers, and the process of aging (Kindo *et al.*, 2014). Further, there have been no detailed *in vitro* studies on antioxidant properties of the plant of *H. incana*. Hence the current study is aimed on the evaluation of Methanol leaf extracts of *H. incana*.

MATERIALS AND METHODS

Plant material collection

Henckelia incana (vahl) Spreng was collected from Gopalswami hills, Western Ghats of Tamil Nadu, South India.

Sample preparation

The methanol extract of the sample leaves (Dried) were prepared according to the method described by Harbone, 1998. The samples were extracted using a Soxhlet apparatus. They were collected and concentrated under reduced pressure at 40°C. The extracts were dried and stored at 4°C in storage vials for experimental use.

Antioxidant activity sample preparation

Methanol extract of *H. incana* was dissolved in DMSO. Different concentration of samples were prepared and used for different assays. Ascorbic acid was used as standard for comparing the activities.

DPPH radical scavenging activity

The free radical scavenging capacity of the evaporated sample of *Henckelia incana* extract was determined using DPPH by Blois (1958). DPPH solution was prepared in 95% methanol. Different concentration (12.5-100 mg/ml) of methanol extract of *H. incana* was reacted with freshly prepared 3.0 ml DPPH solution (0.1mM) and incubated at room temperature. After 30minutes, the absorbance

was taken at 517 nm using a spectrophotometer (Labman UV-Visible spectrophotometer). Ascorbic acid was used as a reference standard. The Control was devoid of the sample. Standard ascorbic acid was treated similar to the extracts. The percentage of inhibition was calculated by following formula.

% of DPPH radical scavenging activity = $(A \text{ of control} - A \text{ of sample} / A \text{ of control}) \times 100$

Total antioxidant capacity by phosphor Molybdenum assay

The spectrophotometric measurement of Total antioxidant capacity (TAC) is based on the reduction of Mo (VI) to Mo (V) by antioxidant compound and the formation of green phosphate / Mo (v) complex at acidic pH (Prieto *et al.*, 1999). Different concentrations (12.5-100 µg/ml) of methanol leaf extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium Molybdate). The tubes containing the reaction solution was incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm. Methanol (0.3 ml) in the place of extract was used as the blank. Ascorbic acid was used as a reference standard.

Reducing power assay

The reducing power of methanol leaf extract of *H. incana* was determined according to the method previously described by Oyaizu (1986). Different concentrations of methanol extracts of *H. incana* were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%) separately. The mixtures were incubated at 50°C for 20 min. Then, 2.5 ml of tri Chloro acetic acid (10%) was added to the mixtures and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank.

Results and Discussion

H. incana is an important genus of the family Gesneriaceae. Survey of literature did not reveal any reference to a previous work on antioxidant of the plant *H. incana*. The present study on *H. incana* was undertaken with the aim to study the antioxidant activity of leaves. Antioxidant contents of methanol extract of *H. incana* leaves were investigated adopting non enzymatic methods (DPPH radical scavenging activity, reducing power and total antioxidant assay).

DPPH radical scavenging activity

The table (1) indicates the free radical scavenging ability of methanol extract of *Henckelia incana* leaves and standard ascorbic acid. Methanol extract of leaf exhibited significant radical scavenging effect of $86.61 \pm 0.47\%$ at $100 \mu\text{g/ml}$ concentration. This value was significant when compared to standard ascorbic acid which showed $94.58 \pm 1.08\%$ scavenging at $100 \mu\text{g/ml}$ concentration. The IC_{50} value of ascorbic acid was 42.58 ± 0.20 . The IC_{50} value of methanol extract was $43.10 \pm 0.073 \mu\text{g/ml}$, which was almost equal to standard ascorbic acid.

Kundan *et al.* (2017) reported the antioxidant activity of *Didymocarpus pedicellata* which show the free radical scavenging activity (DPPH assay) was $7.74 \pm 0.03 \text{ mM AAE/100g}$. DPPH scavenging activity of the methanol extract of *H. incana* showed stronger scavenging activity than *Didymocarpus pedicellata*. The similar work was done by Dasha *et al.* (2013) on chemical composition and antioxidant activity of endemic plant *Heberlea rhodopensis* showed the DPPH free radical scavenging activity of $0.803 \pm 0.007 \text{ mmol TE/g}$. Mihaylova *et al.* (2015) investigated total antioxidant activity of *Heberlea rhodopensis* extracts obtained by pressurized liquid extraction which obtained the DPPH activity as $72.98 \pm 0.18 \mu\text{M TE}^{-1}\text{g DW}$. The methanol extracts of *Henckelia incana* showed greater antioxidant activity than *Heberlea rhodopensis*.

Table 1: DPPH radical scavenging activity of *Henckelia incana*

DPPH radical scavenging activity of <i>Henckelia incana</i>					
Concentration ($\mu\text{g/ml}$)	12.5	25	50	100	IC50
Methanolic extract (% of inhibition)	54.29 ± 0.89	73.86 ± 0.56	83.39 ± 0.78	86.61 ± 0.47	43.10 ± 0.073
Concentration ($\mu\text{g/ml}$)	12.5	25	50	100	IC50
Ascorbic acid (% of inhibition)	35.20 ± 2.67	67.01 ± 1.52	80.42 ± 1.51	94.58 ± 1.08	42.58 ± 0.20

Total Antioxidant Activity

The plant *Henckelia incana* of methanolic extract exhibited significant antioxidant activity. Increase in absorbance indicates increase in total antioxidant capacity. The absorbance increase with concentration and the values are compared with ascorbic acid. The methanol extract exhibited significant absorbance of 1.06 ± 0.04 for 100 $\mu\text{g/ml}$. Ascorbic acid had an absorbance 0.74 at 100 $\mu\text{g/ml}$ concentration (Table 2).

Table 2: Total Antioxidant Activity of *Henckelia incana*

Concentration ($\mu\text{g/ml}$)	12.5	25	50	100
Methanolic extract (Absorbance)	0.30 \pm 0.05	0.46 \pm 0.03	0.73 \pm 0.05	1.06 \pm 0.04
Concentration ($\mu\text{g/ml}$)	12.5	25	50	100
Ascorbic acid (Absorbance)	0.068	0.158	0.313	0.700

Bailey *et al.* (2012) studied the in vitro antioxidant activity of *Rytidophyllum tomentosum*. The total antioxidant activities ranged from 12.50 \pm 0.97 mg/g to 42.10 \pm 0.77 mg/g. Free radicals are conversantly are reactive oxygen species that contribute to aging and degenerate diseases. Radovanovic *et al.* (2015) reported the total antioxidant activity of *Allium porrum* extracts, the result expressed ascorbic acid equivalent per gram of dry extract show that the highest antioxidant activity (128.01 $\mu\text{g AA/g}$ dry extract). The total antioxidant activity of *Henckelia incana* also shows the greatest antioxidant activity.

Reducing Power Assay

The antioxidant activity was done by using reducing power assay which shows the increase in absorbance. High absorbance indicates high reducing power of methanolic extract. Ascorbic acid as control exhibited significant absorbance of 0.14 \pm 0.09 for 100 $\mu\text{g/ml}$ and ascorbic acid had an absorbance 0.762 at 100 $\mu\text{g/ml}$

concentration. Irshad *et al.* (2016) reported the antioxidant activity of steroidal constituents from aerial parts of *Didymocarpous pedicellata* in corresponds to reducing power as compared to gallic acid and ascorbic acid. The reducing power found to be effective and dose dependent. In this present study the methanolic extract of *H. Incana* also showed the similar result. The reducing power of several *Haberlea rhodopensis* leaf extracts of methanol and 70% ethanol was studied by Mihaylova *et al.* (2011). The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. Similarly results also observed in *H. incana*.

Table 3 Reducing power assay of *Henckelia incana*

Concentration	12.5	25	50	100
Methanolic extract	0.05±0.02	0.06±0.02	0.09±0.04	0.14±0.09
(Absorbance)				
Concentration	12.5	25	50	100
(µg/ml)				
Ascorbic acid	0.101	0.109	0.374	0.762
(Absorbance)				

Reference

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**PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE FERN
CHEILOSORIAMYSURENSIS(WALL. EX HOOK.)**

CHING & SHING AND ITS ANTIOXIDANT ACTIVITY

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Abstract

Cheilosoriamysurensis is a medicinal fern used to treat a variety of ailments, with few researches done on its phytochemical makeup and potential bioactivity. The main aim of the study was to support that traditional herbal medicine with active phytochemicals would be a good substitute for allopathic medicine. The methanol extract was tested for preliminary phytochemical analysis and its antioxidant activity. *Cheilosoriamysurensis* exhibited significant reducing power and total antioxidant activity, and its absorbance increased with an increase in concentration of sample. Overall, the findings suggest that this fern could be useful for medical purposes as a source of phytochemicals.

Keywords: *Cheilosoriamysurensis*; antioxidant; medicinal fern; Reducing power: Total antioxidant assay:

Introduction

focuses on locally available plant species and plant-based products, as well as ancient knowledge, traditional medicine can be found all over the world (AwasandDemissew, 2009). Human society faces numerous obstacles, and coping with health problems is a serious societal concern, mainly in growing international locations with restricted resources (Mangambu, 2013). The development in biology and medicine, many people in growing countries do not have sufficient fitness care (Singh and Singh,2012). The world population, especially the rural population in developing countries are still largely dependent on herbal treatments (Islam, 2014).

A number of diseases have become increasingly important in recent times, of which infectious diseases caused by bacteria and cancer are two areas that need immediate attention. Plant sources with diverse natural bioactive ingredients play an important role in curing dreaded diseases (Atanasov, *et al.*, 2015). Many chemical compounds show impressive in vitro activity against pathogenic microorganisms resistant to recent allergenic drugs. The identification and characterization of these lead molecules in terms of their biological activity on the spectrum, potency, toxicity and safety is most needed.

Pharmacological research and traditional medicine have brought many drugs into the international pharmacopoeia. Pteridophytes are immune to many microbial diseases, it is possible that this is one of the reasons for their evolutionary success and the fact that they have been around for over 350 million years. Screening plant extracts for antibacterial, antioxidant, and anti-cancer effects could be beneficial to both humans and plants, given the enormous diversity of Indian medicinal plants and pteridophytes. In recent years, antimicrobial resistance has become a global issue. Because medicinal ferns are biodegradable, harmless and have fewer side effects, it is necessary to screen them for novel bioactive chemicals (Prusti, 2008).

Recently, biological research has concentrated on the medicinal, pharmaceutical, and phytochemical aspects of pteridophytes, which have significant value for medical and industrial uses. Many pteridophyte species are still being studied for possible uses and for the extraction of new active components. Hence, in this work is carried out the preliminary phytochemical analysis and its antioxidant activity of the fern, *Cheilosoria mysurensis*, found in the Western Ghats, Tamilnadu.

Materials and Methods

Plant material

Cheilosoria mysurensis (Wall. ex Hook.) Ching & K. H. Shing were collected from the Gopalaswamy hills, Western Ghats of Tamilnadu, South India. The fern species was identified and authenticated by BSI, Southern circle, Coimbatore, Tamilnadu. A voucher specimen was deposited as herbarium at the Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi.

Sample preparation

The methanolic extract of the fern leaves (Dried) were prepared according to the method described by Harbone, 1998. The sample was extracted using a Soxhlet apparatus. The collected extract was concentrated under reduced pressure at 40°C. The extract was dried and stored at 4°C in storage vials for experimental use.

Qualitative phytochemical Analysis

Test for alkaloids

About 2 g of plant materials were crushed then added 1 mL of ammonia. Furthermore, 10 mL of chloroform was added, then crushed and filtered. The filtrate was added 10 mL of sulfuric acid 2N, shaken vigorously, left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer is taken into a test tube and tested by Meyer reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate indicate the presence of alkaloids (Miles, 1985).

Test for Terpenoid, Steroid, and Saponin:

The methanol extract was concentrated and partitioned with hexane. The soluble extract in hexane was tested with the Liberman-Bourchard reagent. The blue or green color exhibits the presence of steroids and red color for terpenoids. The insoluble residue in hexane is added water and shaken vigorously. The presence of the stable foam for 30 min indicates the existence of saponins, if positive for saponins, the solution was hydrolyzed with HCl and tested with the Liberman-Bourchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoidsaponins (Lajis,1994).

Test for Flavonoid:

The methanol extract was concentrated and partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, subsequently added 0.5 mg of magnesium and HCl 0.5 M. The pink or purple color shows the presence of flavonoids (Halimatussakdiah and Amna, 2016)

Test for Phenol:

Methanol extract tested by Ferric Chloride. Add 3 – 4 drops of FeCl₃ solution into extract, the formation of bluish black color exhibits the phenol compound (Tiwari *et al.*, 2011).

Test for Tannin:

About 0.5 g of methanol extract was boiled in 10 ml of water in the test tube and then filtered. Add a few drops of FeCl₃ 0.1%. Forming of a brownish green or bluish black color indicates tannins (Ayoola *et al.*, 2008)

Test for Glycosides (Keller-Killani Test):

Glacial acetic acid was added into 2 ml. extract and one drop 5% FeCl₃ and conc. H₂SO₄. Reddish brown color appears at the junction of the two liquid layers and the upper layer of bluish green indicates the presence of glycosides (Kokate *et al.*, 2001).

Antioxidant activity

Sample preparation

The methanol leaf extract of *Cheilosoriamysurensis* 1.0 mg was dissolved in 1 ml of DMSO. From the above 125, 250, 500 and 1000µg/ml concentration of sample was prepared and used for different assays. Ascorbic acid was used as standard for comparing the activities.

Reducing power assay

The reducing power of methanolic leaf extracts of *C.mysurensis* was determined according to the method previously described by Oyaizu (1986). Different concentrations of methanol extracts of *C.*

mysurensis was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%) separately. The mixtures were incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixtures and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank.

Total antioxidant capacity by phosphomolybdenum assay

The spectrophotometric measurement of Total antioxidant capacity (TAC) is based on the reduction of Mo (VI) to Mo (V) by antioxidant compound and the formation of green phosphate / Mo (v) complex at acidic pH (Prieto *et al.*, 1999). Different concentrations (125- 1000µg/ml) of methanolic leaf extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution was incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm. Methanol (0.3 ml) in the place of extract was used as the blank. Ascorbic acid was used as a reference standard.

Results and Discussion

Phytochemical analysis:

The preliminary phytochemical analysis results of *Cheilosoriamysurensis* methanol extract was recorded (Table-1). *C. mysurensis* contains Alkaloids, steroids, saponins, phenols tannins and coumarins.

Table-1Phytochemical analysis of methanol extract of *Cheilosoriamysurensis*

Secondary Metabolites	Methanol Extract of <i>Cheilosoriamysurensis</i>
Alkaloid	+
Terpenoid	-
Steroid	+
Saponin	+
Flavonoid	-
Phenol	+
Tannin	+
Glycosides	+

Pradnyaet *al.* (2015) reported phytochemical analysis of four *Cheilanthes* species from northern Western Ghats of India. The methanolic extract of these ferns contains steroids, triterpenoids, reducing sugar, sugars, alkaloids, phenolic compounds, flavonoids, catechins and saponins. In our present investigation methanol extract of *Cheilosoriamysurensis* expressed the positive results for various phytochemical results. Pandey *et al.* (2014) investigated phytochemical analysis of methanolic extract

of *Adiantum* and *Pteris* leaf and stem contains tannins, saponins, flavonoids, and terpenoids steroid terpenoid. Similar results were obtained in the present study.

Damaynatiet *al.* (2019) reported secondary metabolites like alkaloids, flavonoids, carbohydrates, glycosides, phenol, saponin, steroid, tannins were present in the methanol extract of *Aspleniumindicum*, *Lepisorusnudum* and *Microsoriummembranecium*. In the present study methanol extract of *Cheilosoriamysurensis* expressed the positive results for various phytochemical results. Bharti (2018) reported that methanolic extract of *Ampelopterisprolifera* and *Lygodiumflexosum* shows phytochemical compounds of carbohydrates, protein, aminoacids, alkaloids, glycosides, saponins, flavonoids, phenolics and tannins. In the present study methanol extract of *Cheilosoriamysurensis* expressed the positive results for various phytochemical results.

Herinet *al.* (2013) reported the methanolic extract of *Pterisargyreae*, *P.confuse*, *P.vittata*, *P. biaurita* and *P. multiaurita* shows phytochemical compounds of steroids, alkaloids, saponins, flavonoids, triterpenoids, phenolic compounds and tannins. In present study methanol extract of *Cheilosoriamysurensis* results were directly coincided with the above results.

Antioxidant activity

Reducing power assay

The reducing power of *C. mysurensis* methanol extract was shown in **Table 2**. The reducing power of methanol extract of absorbance (0.10 ± 0.01 to 0.03 ± 0.006) increased with concentration of methanol extracts of 12.5 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$. The *C. mysurensis* extract showed potent ferric reducing power at 100 $\mu\text{g/ml}$ concentration. Ascorbic acid had increase in absorbance value indicate increase in Reducing power capacity.

How yeelai *et al.* (2011) evaluated that antioxidant activities of the methanolic extracts of selected ferns in Malaysia. In this FRP method measured the ability of an antioxidant to donate electron to Fe (III) resulting in the reducing of Fe^{3+} / ferricyanide complex to Fe^{2+} complex, which would be monitored at 700 nm. Results we expressed as Gallic acid equivalent (GAE)/100 g leaves. The higher the FRP value, the greater is the reducing power of tested compound, thus greater the antioxidant activity. In the present study, the results indicate that the reducing ability of the extracts increased with the concentration. The result of *C. mysurensis* was significant. The reducing power of *C. mysurensis* extracts in the descending order. The reducing capacity of ascorbic acid was found to be higher than the extracts at each concentration points.

Gupta *et al.* (2014) reported the In vitro antioxidant activity of methanolic extracts of some ferns from Mawsynram of Meghalaya, India. The reducing ability of *Aleuritopterisflava* extracts was determined with ascorbic acid equivalent. Higher ascorbic acid equivalent value indicates higher reducing capacity

of sample, thus greater antioxidant potential. In the present study, the results indicate that the reducing ability of the extracts of *C. mysurensis* was increased with the ascorbic acid. Pandey *et al.* (2014) reported that antioxidant potential of methanolic extract of Adiantum and Pteris ferns. Analysis of leaf of Adiantum and Pteris ferns has a more 33 reducing power compared to their stem. There is a significant increase in absorbance of the reaction mixture indicates reducing power. In the present study, the result indicate that the reducing ability of the extracts of *C. mysurensis* was increased with the absorbance.

Table 2: Reducing power assay of *C. mysurensis*

Concentration	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Methanol extract (Absorbance)	0.03 ± 0.006	0.07 ± 0.03	0.09 ± 0.01	0.10 ± 0.01
Concentration	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Ascorbic acid (Absorbance)	0.101	0.109	0.374	0.762

Total antioxidant capacity assay

Increase in absorbance indicates increase in total antioxidant capacity. The methanolic extract of *C. mysurensis* exhibited significant activity and its absorbance increased with increase in concentration and the values were in comparison with ascorbic acid. The methanol extract exhibited significant absorbance of 2.1±0.14 for 100µg/ml. Ascorbic acid had an absorbance 0.74 at 100 µg/ml. Jose *et al.*(2017) studied the antioxidant effects of hexane, ethyl acetate, and methanolic extract of *Pyrrosiaheterophylla*. The assay is based on reduction of phosphate – molybednum (IV) to phosphate molybednum (v) by various concentrations (50,100,200,400 and 800µg/ml). The result of *C. mysurensis* also show smiliar activity.

Salehaet *al.*(2014) reported the total antioxidant activity of fern Diplaziumesculentum. The total antioxidant potentials of DECH and DEM showed higher antioxidant activity compared to standard ascorbic acid. In the present study, methanolic extract of *C. mysurensis* also show the same result. Selvarajet *al.* (2015) revealed the antioxidant effects of methanolic extract of *Azollamicrophylla*. From this analysis, it was evident that antioxidant increases with increase in concentration. The result of *C. mysurensis* exhibited significant activity and also antioxidant increases with increase in concentration. The total antioxidant capacity of *C. mysurensis* was shown in Table 3.

Table 3: Total antioxidant capacity by phosphomolybdenum of *C. mysurensis*

Concentration	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Methanol extract (Absorbance)	0.4 ± 0.04	0.7 ± 0.05	1.2 ± 0.02	2.1 ± 0.14
Concentration	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Ascorbic acid (Absorbance)	0.068	0.158	0.313	0.700

Conclusion

The chemical composition, antioxidant, and cytotoxicity activities of the methanol extract of *C. mysurensis* from the Western Ghats of South India are reported for the first time. According to the findings of this study methanol extract of the fern contains contain Alkaloids, steroids, saponins, phenols tannins and coumarins. Furthermore, the findings demonstrated that the *C. mysurensis* methanol extract contains powerful antioxidant activities, as well as being a promising source for developing future antioxidant and anticancer medications.

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REVISITING TEACHING METHODOLOGY IN BIOLOGICAL SCIENCES ON NEP 2020 PERSPECTIVE

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Abstract

There are very few studies concerning the importance of teaching methods in biology education and environmental education including outdoor education for promoting sustainability at the levels of primary and secondary schools and pre-service teacher education. The material was selected using special keywords from biology and sustainable education in several scientific databases. The foci of the analyses were teaching methods, learning environments, knowledge and thinking skills, psychomotor skills, emotions and attitudes, and evaluation methods. Additionally, features of good methods were investigated and their implications for teaching were emphasized. In total, 22 different teaching methods were found to improve sustainability education in different ways. The most emphasized teaching methods were those in which students worked in groups and participated actively in learning processes. Research points towards the value of teaching methods that provide a good introduction and supportive guidelines and include active participation and interactivity for learning biology. The New education Policy (NEP- 2020) which opens a wide arena to learn the biological science with many educational softwares, IOTs hence the transformation of teaching has become imaginary to reality among the students and faculties. The present paper also establishes the available resources that enhance the Biological science education to next level in forthcoming years.

Keywords: Biology education; Environmental education; NEP 2020, IOTs

Introduction

Education is one of the goals for the future is the construction of a sustainable society [Gladwin, 1995]. A sustainable society is considered to be a society that has reached sustainability through a process called sustainable development. Sustainable development as a concept is heavily context-dependent in social, cultural, and environmental situations [Kopnina, 2012]. Brundtland's report defines sustainability as –development that meets the needs of the present without compromising the ability of future generations to meet their own needs [United Nations Report, 1987]. According to [Diesendorf, 2000], this definition emphasizes the long-term aspect of the concept of sustainability and introduces the ethical principle of achieving equity between the present and future generations. It does not mention the natural environment explicitly, focusing only upon human needs or wants. However, the report makes it clear that these –needs include the conservation of the natural environment. More recently, it has been given a broader definition which conveys that there are three principal dimensions: an ecological, economic and social. In the teaching and learning of sustainable development, the ecological dimension refers to the natural one and includes all living things, resources and life-supporting systems. Its goal is conservation. The economic dimension comprises jobs and income, and its goal is appropriate development. The social dimension involves people living together. Its goal is peace, equality and human rights. In addition to these three dimensions, there is also a fourth one, the political dimension. It has to do with politics, policy and decision-making as a goal of democracy [Fien, 2009]. The ecological issues are important in biology education, e.g., in Finland, Sweden and Denmark, they form the core content in the curricula of biology for basic education. All biology curricula emphasize different biotopes and ecosystems, lifecycles of plants and animals, and life-supporting processes, such as photosynthesis, respiration and biodiversity, but they do not mention the other dimensions of sustainability [Skolverket, 2000]. For this reason, we stress the ecological aspect in this study. Our aim is to find out and describe useful teaching methods in biology education and sustainability education (SE) including outdoor education (OE) for promoting sustainability in primary and secondary schools and teacher education. As far as we know, there are no previous studies from these perspectives.

An ongoing debate over the last three decades has been how the role of education should be conceptualized when creating sustainability and a sustainable future. Sustainability and a sustainable future are here understood as the goals of sustainable development. The relationships between environmental education, education for sustainable development, and sustainable development education have been discussed. Environmental education and education for sustainable development are interpreted in different ways around the world, according to context [Wessellink, 2011]. Some authors argue that education for sustainable development is a part of environmental education [Mckeown, 2003] or a perspective of environmental education [Suave, 2005], or that environmental education has developed into education for sustainable development [Eilam, 2010]. In Agenda 21, it has been stated that environmental education is a continual, life-long learning process to raise public awareness and action globally, nationally and locally in

every area in which humans impact the environment. Important distinctions between the goals of environmental education were made by [Lucas,1979]—in,|| –about|| or –for|| the environment—in order to avoid misunderstandings about the intended type of environmental education.

According to [UNESCO, 2016], education for sustainable development is about enabling people to constructively and creatively address present and future global challenges and create more sustainable and resilient societies. Learning in education for sustainable development often includes only knowledge, values and theories related to sustainable development. However, it also means –learning to ask critical questions; learning to clarify one’s own values; learning to envision more positive and sustainable futures; learning to think systematically; learning to respond through applied learning; and learning to explore the dialectic between tradition and innovation|. Thus it offers learners a context for developing active citizenship and participation, embracing the complexity of the interdependencies of ecological, societal, and economic systems. The overall goal of the UN Decade of Education for Sustainable Development (2005–2014) was to integrate the principles, values, and practices of sustainable development into all aspects of education and learning [UNESCO, 2009]. In Finland, sustainability is included in the curriculum for basic education at all educational levels. How this has been done is described in more detail in another article of this special issue [Wolff, 2016]. Sustainable development education again is based particularly on environmental and ecological sciences and focuses on the interaction between ecological and social systems. It encourages students to critically reflect on the ideas of sustainable development and the values that underlie them, and to create solutions to achieve concrete goals in a variety of unpredictable situations [Dale, 2005]. As noted above, both environmental education, education for sustainable development, and sustainable development education share a vision of quality education and a society that lives in balance with Earth’s carrying capacity. They are thus integrated and represented in all dimensions of sustainable development. In this study, we use the term sustainability education (SE) [Sterling, 2010] because it catches all forms of environmental education, education for sustainable development, and sustainable development education.

Methods

The study is a qualitative survey with quantitative features [Morse, 2010 and Collins, 2010]. At first, we examined the selected 17 journals concerning biology education and SE. They included in total 29 articles that mentioned teaching methods. From these, we selected 16 journals with 24 articles to be analyzed in detail.

In the analysis, we followed the method of qualitative content analysis. Inductive content analysis was used to analyze teaching methods, learning environments and features of useful teaching methods and implications. Deductive content analysis was used to analyze psychomotor skills, emotions and attitudes, knowledge and thinking skills and also evaluation methods [Mayring, 2000].

In order to ensure the reliability of the process, all three members of the research team first conducted the selection of information units, the categorization and the subsequent analysis independently. The analysis process was dialogical by nature. The final decisions were made through e-mail discussions where each researcher argued why the content of the article should be placed into a certain category or categories. The discussion continued until consensus was reached and clear arguments were found. The generalizability of results relates to the selection of analyzed data. To ensure that our categorization decisions were based on comprehensive understanding of the article, we decided to read the whole article before categorizing it. We also based our analysis on what the authors of the articles had explicitly written rather than what we in some cases thought we could read between the lines as being the authors’ intentions. As such decisions always include elements of subjective interpretation, joint discussions about each article were essential in deciding which aspects of the instructional process the article emphasized. This procedure ensured that decisions were not based on a single person’s first impression of an article but on well-argued joint discussions. Because of the dialogical nature of the analysis, we did not see a need for calculating an inter-rater reliability. Researcher triangulation was an essential part of our analysis process. Our research group consisted of experts from biology education, environmental education, sustainable development education, and educational sciences, and all researchers are experienced teacher educators and researchers.

Findings and Discussion

This article mainly concern over the teaching methods in relation to other issues in the studies of biology and sustainability education. Teaching methods can be seen as objective-oriented activities and flow of information between teachers and students. Studies of teaching methods are important because teaching methods influence all types of learning in the cognitive, affective and psychomotor domains [Karami et al., 2012, Prince, 2006]. The choice of teaching methods depends on what kind of teaching approach is preferred. Traditional instruction in biology is deductive and comprises the principles and methods used for instruction to be implemented by teachers to achieve the desired learning or memorization by students. In this kind of teacher-centered approach to learning, teachers are authorities and students’ primary role is to passively

receive information through lectures and direct instruction. Learning is measured using objectively scored tests and assessments [Prince, 2006 and Eisner, 2004]. Alternative teaching approaches are inductive where instruction begins e.g., with observations, experimental data to interpret or a real-world problem to be solved. In this student-centered approach to learning, teachers and students play an equally active role in the learning process. The teacher's primary role is to coach and facilitate student learning and overall comprehension of material. Learning is continuously measured using both formal and informal forms of assessment, including group projects, student portfolios, and class participation [Prince, 2006]. The selection of teaching methods is affected by the learning objectives. The clearly specified learning objectives also provide the goals at which the curriculum is aimed, they facilitate the selection and organization of content, and they make it possible to evaluate the outcomes of the learning [Eisner, 2004]. Several good features are emphasized in the analyzed teaching methods and have implications especially for developing curricula and teaching for sustainability.

Learning Environments

Since learning environments have been developed to support the selected teaching methods, they both have an effect on learners' achievements. The most often used learning environment were classrooms, which were mentioned in 22 out of 24 articles. Introductions, guidelines, and discussions concerning learning experiences and results of observations and experiments were often carried out in the classrooms, in addition to traditional teacher presentations and inquiries. Outdoor and field environments were mentioned in 14 articles. Different visiting places, such as museums, gardens, and nature parks, were the third most common learning environments (mentioned in 11 articles). Such places appear to be good learning environments because students' learning results are significantly better when they get first-hand experiences and studies in authentic learning environments [Smeds, 2015].

Laboratory environments were found to be more effective and although laboratories are places where students can meet real scientists and learn how research is done. In school laboratories, students can develop their experimentation skills when planning and carrying out small studies. The internet and electronic discussion forums were mentioned in two articles.

Features of Useful Teaching Methods and Implications

The article gives a clear endorsement for the provision of a certain kind of biology education approach. This research emphasizes the value of teaching methods which have a good introduction and supportive guidelines and include active participation and interactivity. The results support Rickinson's research. First-hand experiences, locality and place-based education, and OE are also emphasized. Conversely, systematic teaching of sustainable development, teachers as role models, continuing development of EESD (Environmental Education for Sustainable Development), positive feedback, and whole-school approaches were not popular (mentioned only twice). Moreover, neither continuous teacher and staff education nor differentiation were popular, having been mentioned only in one article together with the ideas that SE should be taken into account at all education levels, and that there should be enough time for SE. The reason could be similar to those that Rickinson reported in his study, e.g., that the aims of SE are not always realized in practice, the different types of barriers faced by individual students and teachers in learning and teaching SE, and familiarity with the SE setting [Rickinson, 2004].

Conclusion

The study aimed to identify and describe useful teaching methods in biology education and sustainability education (SE) including outdoor education (OE) for promoting sustainability. Although our analyses of recent research on teaching methods and their evaluation included several details, a holistic view of the educational processes is needed for the understanding of all effects. All teaching methods are, of course, context- and subject-dependent, and cannot therefore be arranged as a list of the most or least effective methods. The analyses, however, provide ideas of how to use these methods together for promoting sustainability aspects in teaching, and also of how to evaluate the whole process for the purpose of curricula development. The study emphasizes especially the value of inductive teaching methods with student-centered approaches in authentic environments with first-hand experiences. Like previous research the analyses also emphasized fieldwork and field trips, including problem-based activities, as factors increasing students' interest in and knowledge of sustainability. Fieldwork appeared to have positive effects also on students' attitudes and behavior concerning sustainability. Students' relationships with and connectedness to nature, environmental consciousness, and interest in biology are all important factors in any attempt to create a sustainable future. Reviewing all the parameters the science is getting more popular and changing itself into an applied nature and the development in life science is also unavoidable. In this connection with the New Education Policy it is necessary to provide more field oriented study with live experiments, practical demonstrations. So students may get benefitted in learning new things.

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***IN VITRO* ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACTS OF
Henckelia Incana (VAHL) SPERNG.**

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ABSTRACT

The study focuses on the antioxidant capabilities of an endemic herb *Henckelia incana* (Vahl) Spreng (syn : *Didymocarpus tomentosa* Wight). This is an unexplored plant species belongs to the family Gesneriaceae, collected from Gopalswami hills, Western Ghats of Tamilnadu. The leaves of *H. incana* were dried; powdered and methanolic extract was extracted using soxhlet apparatus. Antioxidant activities of the plant extracts was determined by *in vitro* methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Total Antioxidant assay by phosphomolybdenum and Reducing power assay. All assays were carried out by the methanolic extracts of sample dissolved in DMSO. Ascorbic acid was used as standard. DPPH scavenging of leaf extract was significant and the IC₅₀ value was calculated as 43.10±0.073µg/ml. Dose dependent Reducing power and total antioxidant capacity was observed significantly. This study reveals that *H. incana* extracts have a high antioxidant capacity and may be useful for nutritional and therapeutic purposes.

Keywords: *Henckelia incana*, DPPH, Free radicals, Total Antioxidant activity, Reducing power assay

EFFECT OF TRADITIONAL HERBAL PLANT EXTRACTS ON GLUCOSE LEVEL IN YEAST CELLS FOR DIABETIC DISORDER

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Abstract

In the present study, the following herbal plants were tested for their anti-diabetic activity. The selected study species such as *Justicia tranquebariensis* L.f. belongs to the family Acanthaceae, *Momordica charantia* L. belongs to the family Cucurbitaceae and *Sesbania grandiflora* (L.) Poiret belongs to the family Fabaceae. The methanolic extracts were prepared from the leaves of study species by soxhlet extraction method. Hence the anti-diabetic activity was determined by glucose uptake in yeast cells. The yeast cells were suspended in various concentrations of plant extract (10, 25, 50, 75 and 100 %) with two different concentrations of glucose (50 mg/ml to 100 mg/ml). The plant extract enhances the yeast cells to take in the glucose and the amount of glucose uptake by yeast cells was estimated by spectrophotometrically at 540 nm. All the extracts of plant species are exhibit potent anti-diabetic activity. In addition, the results identified the concentration of plant extracts increases the percentage of glucose uptake also increases. The overall study results, it is concluded that the maximum percentage of glucose uptake was observed in 100 % concentration of extracts at both level of glucose concentrations. The results revealed the maximum percentage of glucose uptake 93.96 for *J. tranquebariensis* followed by 93.70 for *M. charantia* was observed at 50 mg/ml glucose concentration respectively and the maximum percentage of glucose uptake 93.66 for *S. grandiflora* was observed at 100 mg/ml glucose concentration. The present study provided results to justify the traditional claim of herbs for antidiabetic activity. Hence, the further extended the work to confirm anti-diabetic activity by acute toxicity studies and on *in vivo* models.

Keywords: Herbal plants, anti-diabetic activity, glucose uptake

**TAXONOMIC SIGNIFICANCE ON MORPHOLOGICAL AND ANATOMICAL
CHARACTERS OF *Cardiospermum Halicacabum* L. and *Cardiospermum Corindum*
L. (Sapindaceae)**

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ABSTRACT

The taxonomic significance on morpho/anatomical characters for the selected study species such as *Cardiospermum halicacabum* L. and *Cardiospermum corindum* L. has clearly explained and recorded with additional evidences. This study was mainly focused on their similarities and differences of morphological and anatomical characters observations between the species. The proper descriptions with prominent characters are discussed for the proper identification of closely related misidentified study species. In overall results the study species *Cardiospermum halicacabum* was morphologically distinguished from *C. corindum* by leaf, stem, internode, and fruit features. The anatomical characters of these two species were differentiated with some prominent characters. Especially for *Cardiospermum halicacabum* the presence of anamocytic type of stomata with diffused in number, stem pentagonal, having 5 short blunt ridges and the xylem vessels are diffused and small in size in root. Whereas in *C. corindum* it was observed dense anamocytic type of stomata, hexagonal stem having 6 short ridges and the xylem vessels in root are dense and large in size. These characters could be the additional taxonomic evidence than the available floras descriptions. It may help to proper identification of closely related allied plants and also it is an important tool in studying evolutionary history and ecological relationships between the species.

Keywords: morpho/anatomical, misidentified species, *Cardiospermum* sp., additional taxonomic evidence

TAXONOMIC SIGNIFICANCE ON MORPHOLOGICAL AND ANATOMICAL CHARACTERS OF *CARDIOSPERMUM HALICACABUM* L. AND *CARDIOSPERMUM CORINDUM* L. (SAPINDACEAE)

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ABSTRACT

The taxonomic significance on morpho/anatomical characters for the selected study species such as *Cardiospermum halicacabum* L. and *Cardiospermum corindum* L. has clearly explained and recorded with additional evidences. This study was mainly focused on their similarities and differences of morphological and anatomical characters observations between the species. The proper descriptions with prominent characters are discussed for the proper identification of closely related misidentified study species. In overall results the study species *Cardiospermum halicacabum* was morphologically distinguished from *C. corindum* by leaf, stem, internode, and fruit features. The anatomical characters of these two species were differentiated with some prominent characters. Especially for *Cardiospermum halicacabum* the presence of anamocytic type of stomata with diffused in number, stem pentagonal, having 5 short blunt ridges and the xylem vessels are diffused and small in size in root. Whereas in *C. corindum* it was observed dense anamocytic type of stomata, hexagonal stem having 6 short ridges and the xylem vessels in root are dense and large in size. These characters could be the additional taxonomic evidence than the available floras descriptions. It may help to proper identification of closely related allied plants and also it is an important tool in studying evolutionary history and ecological relationships between the species.

Keywords: morpho/anatomical, misidentified species, *Cardiospermum* sp., additional taxonomic evidence

INTRODUCTION

Plant identification refer to assigning a plant for a particular taxonomic group and ultimately to the species. Misidentification of closely related plant

species in certain groups of plants may be due in the lack of taxonomic information. This could be as a result of insufficient information on taxonomic characters and overlapping or superimposition of delimitation characters either between the genera or amongst species which is as a result of visible similarities and habits (Ibrahim *et al.*, 2015).

Comparative study of plant structure, morphology and anatomy has always been the backbone of plant systematics, which endeavors to elucidate plant diversity, phylogeny and evolution. The second half of the 20th century was a fascinating period in which systematics and structural studies benefited greatly from new techniques and methods (Endress *et al.*, 2000).

Macro/micro morphological features of leaves, stems, and roots have played important roles in plant taxonomy, especially at the generic and species levels. Studies in this field have attracted the attention of plant morphologists and systematics to resolve taxonomic conflicts in different groups of plants (Sonibare *et al.*, 2014). The foliar epidermal structure has been one of the most important taxonomic characters in biosystematics and most taxonomic studies are based on the studies of the leaf epidermis. In ultra-structures such as epidermal cells, stomata, and trichomes, their sizes, lengths, distribution, orientation, and frequency are the most significant characters in taxonomy as well as phylogeny (Albert and Sharma, 2013).

Anatomical data have been widely used as a taxonomic tool only after the nineteenth century. Anatomical studies have great implication for clarification of taxonomic, ecological and evolutionary relationships in higher ranks of classification. However, in some plant families, several anatomical traits are great value for application at both generic and sub-generic levels (Jones and Luchsinger, 1987). Auguste Mathieu is one of the pioneer taxonomists, who used features of wood anatomy in the taxonomic description of forest plants species in *Florae Forestiere*. Later, another taxonomist Solereder discussed the systematic value of anatomical structures in dicotyledons in his classic book 'Systematics Anatomic der Dicotyledonen'.

Anatomical evidence is systematically useful in a number of ways that is (i) this taxonomic method can be well used in the identification of fragmented materials of biological specimens. (ii) The study of anatomical demonstrations is often useful when there is no use of morphologies in the early identification of herbarium materials. (iii) Anatomical data have proven to be very useful in detecting evolutionary trends and interrelationships of taxa at and above the species level and at higher taxonomic categories. They are very useful to determining relationships between different genera, species, families, orders and

other taxonomic categories. Also the anatomical features have played an important role in elucidation of phylogenetic relationships.

The species of *Cardiospermum* is large genus of tropical American herbaceous vines (Sapindaceae) having alternate biternate leaves, coarsely serrate leaflets, small white flowers and an inflated capsular fruit. In this genus approximately 14 species in the soapberry family. Mostly tropical or subtropical, with a few genera extending to sub-temperate zones; 141 genera and about 1,900 species. Most genera of Sapindaceae are predominantly medium-sized to large emergent trees or erect shrubs, less often they are tendrilled lianas or understory palm-like treelets, exceptionally sub-shrubs or scandent. Vast majority of Sapindaceae genera have trilocular fruits, although many also have bilocular, unilocular, or sometimes quadrilocular fruits. (Sapindaceae Juss. (1789), *Aceraceae* Juss. (1789), Hippocastanaceae. Rich. (1823). Pollen morphology of Sapindaceae holds promise for the recognition of taxa of this cosmopolitan family, which is well distributed in tropical regions and possesses great morphological diversity (Talita Kely Bellonzi, 2020).

Hence the present study was carried out on *Cardiospermum halicacabum* L. and *Cardiospermum corindum* L. were selected to provide the proper taxonomical evidence through morpho/anatomical observation. These additional key characters may fulfil the lacuna towards existing taxonomic description.

MATERIALS AND METHODS

Selection and collection of study plants

The selection of study plants based on lesser taxonomic evidence and commonly misidentifying closely related allied species such as *C. halicacabum* L. and *C. corindum* L. The materials of the plants were collected from Pollachi and surrounding areas, Coimbatore, Tamil Nadu. The Altitude lies under on 293 metres MSL, 10.660207 for Latitude and 76.996727 for Longitude.

Identification of plants

The study plants were identified with using Flora of Presidency of Madras (Gamble, 1935) and further the species were authenticated by Dr. P. Sathishkumar, Assistant Professor, PG department of Botany, NGM College, Pollachi, Coimbatore.

Morphological studies

The following vegetative, reproductive morphological characters was observed. Vegetative (micro/morpho) morphological characters like leaf, stem and root's shape, size, length, width and modifications of stem, leaf hairs, trichomes and glands etc. are observed and stomatal types were also identified in leaf.

The reproductive (micro/morpho) morphological characters like inflorescence, calyx, corolla, androecium and gynoecium with its size, shape and types etc. were observed. The fruit and seed ornamentation with their characters are also studied.

Anatomical studies

Preparation of plant materials:

The collected plant materials were washed with ethanol for removal of dust, dirt and biotic and abiotic substances from external factors.

Preparation of FAA and fixing process

The fixative was prepared by following ratio ethyl alcohol (95 %) 50 ml, Glacial acetic acid 2.5 ml, Formaldehyde 5.5 ml and distilled water 42 ml (Toji Thomas, 2004).

The healthy plant materials was collected separately like stem, leaf and root and stored immediately in FAA solution. After 24 hours, the fixed materials are stored in 50 % ethanol for further studies.

Sectioning process

Free hand sectioning of stem, petiole, leaf and root for selected study plant using razor blade the most transparent and thinnest cross sections was collected carefully and dehydrated with ethanol series (25 %, 50 %, 75 %, 95 % and absolute alcohol).

Staining process

The anatomical sections were stained with double staining method (Toji Thomas, 2004). The selected sectioning species were stained with Safranin for 3 min and washed with 75 % of ethanol for 1 min and stained with methyl blue for 1 min and washed with 90 % of ethanol.

Slide preparation

The sectioned thin slices were mounted in clean dry slides with 50 % glycerine and observed under compound microscope.

Epidermal and stomatal study

The freshly collected leaves were taken and peeled both abaxial and adaxial sides and it was stained with Safranin and mounted on slide for stomatal study. The leaf surface was observed their epidermal and stomatal characters under compound microscope.

RESULT AND DISCUSSION

The taxonomic significance on morpho/anatomical features for the selected study plant species such as *C. halicacabum* L. and *C. corindum* L. has clearly explained and recorded with additional evidences. This study was mainly focused on the similarities and differences of morphological characters

(vegetative and floral) and the anatomical features (leaf, petiole, stem and root) between the species (Figure. 1-10). The proper descriptions with prominent characters are discussed below for the proper identification of closely related misidentified study species.

Morphological studies

C. halicacabum L. - Annual, creaper, stem light green, frequently 5 angled, glabrous, fibrous; Internodes 4-7 cm. long; leaves opposite, biternate, triangular, compound, glabrous, acute tip, apically trifid, unevenly lobed leaf, veniation arise from mid vein and ends in the leaf margin. Mid vein prominent in adaxial, pulvinous leaf base. lamina bright green with a paler under surface, only the main veins raised, apex acuminate, margin incised-serrate, base attenuate; stipules 2 minute caducous scales. Leaf tip Sharp without mucornation; Inflorescence umbrellate cyme, 3 flowers 1 mature and 2 young buds. Inflorescence a reduced complex axillary corymbose thyrs, abortively 3-flowered; peduncle 4–9 cm. long, very sparsely puberulous, multi-bracteate, bract modified tendril on peduncle, flower zygomorphic, complete, white flowers, calyx 4 lobed, (2+2 unevenly sepal) pale green .sepals yellow-green, unequal, the outer \pm round, 1 mm. diameter, the inner larger and ovate, 2–3 mm. long, 1–2 mm. wide; corolla 2 rows (4+4) (2 inner are yellow in colour. Petals white, cream, greenish or yellow, elliptic to obovate, 2–3 mm. long; appendages yellow at tip, hairy; disk of 2 curved elements 1 mm. long; stamens dorsiventral, ditheous, didenmous stamens 8 (4 long 4 short) filament hairs. Stamens free; filaments compressed, 2 mm long, hairy; anthers 0.5 mm long; pistil trifid, white, long triangular ovary, green in colour, trichomes present. pistillode present in male flower. Ovary obovoid, 2-3 mm long, hirsute; style short, pubescent; stigma 3-fid; staminodes 8 in the female flower; Fruit capsule, persistent calyx and stigma on fruit, hairy margin, dehiscing into 3 segments, separated by white papery septa axial placentation; Seeds globose, black, each carpels single seed totally bears 3 seeds, white heart shaped (hilum large) surface on their seed; Pollen triangular, triporate type (Figure.1, 3-9).

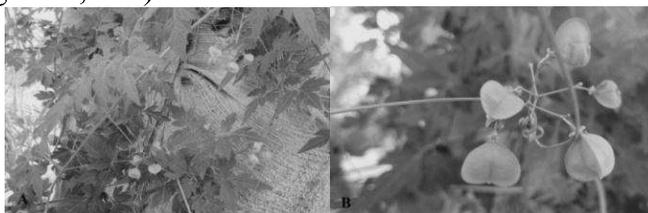


Figure 1. A. Habit of *Cardiospermum halicacabum* L. B. Twig with fruits

C. corindum L. - Annual, creaper, stem light green, slightly woody climber, densely puberulous; internodes 8 cm. long; bract modified into tendril,

apically trifid, opposite to leaves; leaves biternate, triangular leaf, glabrous, main leaflet stalk short, lower leaflet sessile, mostly densely velvety pubescent. Leaves pinnate to biternate; petiole 0.5–3(4.5) cm. long; rhachis 1–3 cm. long. Pulvinous leaf base. Leaf tip Sharp with mucornation. Mid vein prominent in adaxial, wide, acute to acuminate and apiculate at the apex, attenuate-decurrent to truncate or cordate at the base, crenate to serrate or deeply lobed. Lamina bright green with a paler under surface, only the main veins raised, apex acuminate, margin incised-serrate, base attenuate; stipules 2 minute caducous scales; Inflorescences corymbose to paniculate, axillary many-branched, numerous-flowered corymbose thyrses; peduncle 6 cm. long, pubescent, multibracteate; flower zygomorphic, complete, white flowers 4–6 mm. long, sepals unequal; outer broadly elliptic to ± round, 1.5–2 mm. long, 1.5 mm. wide, inner elliptic, 3–5.5 mm. long, 2–3 mm. wide. sepals with lateral ones c. 1 mm. long, anterior and posterior ones c. 3 mm. long, ovate, petals white or cream, elliptic, 4–6 mm. long, 2–3.5 mm. wide; petals 4–5 mm. long, 2 anterior ones ovate with small petaloid appendages, 2 posterior ones ovate bearing recurved appendages with clavate apices covered with patent hairs which cause them to cohere, stamens 8, with filaments 3–4 mm. long, pilose. Stamens with the filaments of the inner ones slightly shorter; filaments free; ovary 2–3 mm. long; style 0.5–1 mm. long; stigmas ± 0.5 mm. long. Ovary hirsute, trifid, white, long triangular ovary, green in colour, trichomes present. pistillode present in male flower. Fruit yellow-green (balloon like) turning greenish brown to reddish or dark brown, obtetrahedral, 1–3.5 cm. long, 1.8–4 cm. wide, dehiscent into 3 segments, at first densely puberulous or pubescent. Seeds black, globose, 5 mm. diameter, with a reniform or crescentic hilar area, 3–4 mm. wide. Seed 5 mm. in diam., reniform; Pollen spherical, triplicate (Fig 2, 4-9).

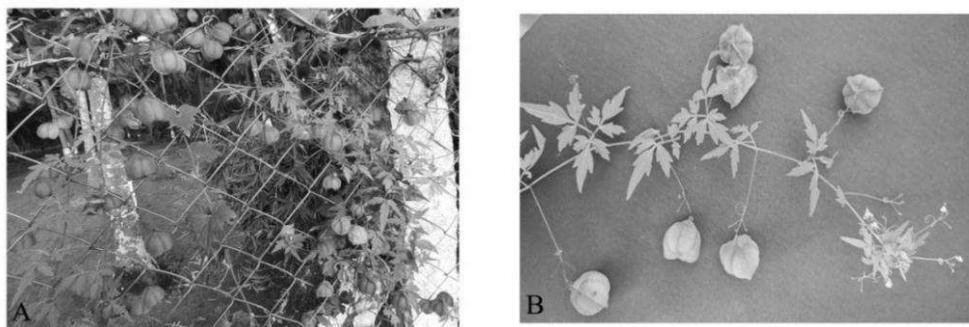


Figure 2. A. Habit of *Cardiospermum corindum* L., B. Twig with fruits

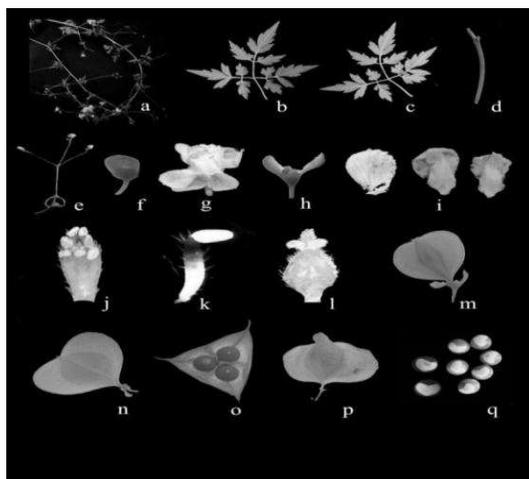


Figure 3. Morphological structures of *Cardiospermum halicacabum* L. – a) Habit, b) Adaxial leaf, c) Abaxial leaf, d) Stem, e) Inflorescence, f) Flower Bud, g) Flower, h) Calyx, i) Corolla, j) Androecium, k) Stamen, l) Gynoecium, m) Young fruit, n) Mature fruit, o) C.S of ovary, p) Fully matured fruit, q) Seeds.

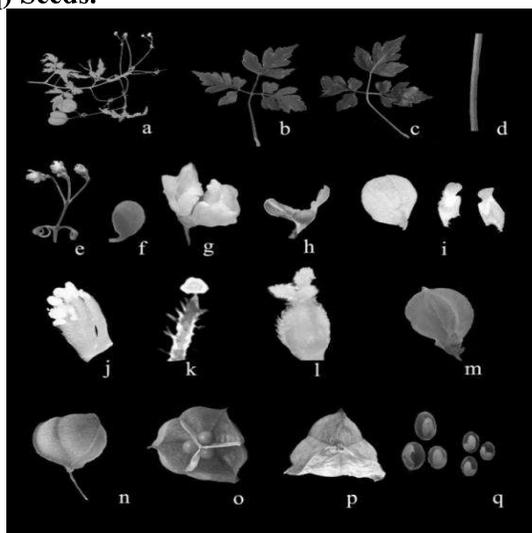


Figure 4. Morphological structures *Cardiospermum corindum* L. – a) Habit, b) Adaxial leaf, c) Abaxial leaf, d) Stem, e) Inflorescence, f) Flower Bud, g) Flower, h) Calyx, i) Corolla, j) Androecium, k) Stamen, l) Gynoecium, m) Young fruit, n) Mature fruit, o) C.S of ovary, p) Fully matured fruit, q) Seeds.

Anatomical studies

Cardiospermum halicacabum L.

Leaves: The adaxial part of midrib is thick and pyramid like and the abaxial part is semicircular with undulate outline. The midrib possesses thick, distinct epidermal layers of fairly large squarish, thick walled cells. The outer epidermis is single layer. The upper epidermis bears the unicellular trichomes. It

is cluster of angular collenchyma. The lower epidermis bears the 2-3 layer of angular parenchyma. The vascular bundles is very prominently found in central axis. The vascular bundles is collateral close type. In which wide circular thin walled xylem elements and a thick band of phloem elements (Figure 5).

Stomata: The anomocytic type of stomata, diffused in number. No specific subsidiary cells are seen. The guard cells are elliptical in shape (Figure 10).

Stem: The stem is pentagonal, having 5 short blunt ridges. The epidermal layer is small spindle shaped cells with cuticle. The ridges with large parenchyma tissues. Sclerenchyma is running around the stem. The vascular cylinder is angular in outline. The phloem surrounds the xylem. The xylem consists of wide and narrow vessels with xylem fibres. The pith is present at the centre, which consist of parenchyma cells (Figure 7).

Root: T.S. of the root shows circular margin with minute trichomes. The outermost layer is epidermis followed by cortex. Secondary cortex is made up of cluster of parenchyma cells. The vascular bundle is found below the cortex. The vascular bundle consists of xylem and phloem. The phloem surrounds the xylem. It is endarch type, where protoxylem faces the centre and metaxylem faces the periphery. The medullary rays are present between xylem and phloem. The xylem vessels are diffused and small in size (Figure 8).

Petiole: C.S. of the petiole is rhomboidal shaped, recurved downwards in abaxial side with presence of protruding structure on both sides. The abaxial side is U shaped. The outermost layer is epidermis, which consist of unicellular trichomes. The cortex is present below the epidermis, which is followed by sclerenchymatous. The vascular bundle is 6 in number collateral closed. The xylem is exarch in nature. The pith is at centre with cluster of cells (Figure 6).

***C. corindum* L.**

Leaves: The midrib's adaxial part pyramid like and the abaxial part is semicircular outline. The midrib possesses thick distinct epidermal layers of fairly large squarish thick walled cells. The epidermis is single layer. The upper epidermis bears unicellular trichomes. It is 4-5 layer angular collenchyma. The lower epidermis bears the 3 layer of angular parenchyma. The vascular bundles is very prominent found in central axis. The vascular bundles is collateral close type. In which wide circular thin walled xylem elements and thick band of phloem elements (Figure 5).

Stomata: The anomocytic type of stomata, dense in number. The guard cells are elliptical in shape. No specific subsidiary cells are seen (Figure 10).

Stem: The stem is hexagonal, having 6 short blunt ridges. The epidermal layer is spindle shaped cells with cuticle. The epidermis is followed by

sclerenchyma layer of cells. The vascular bundle phloem surrounds the xylem. Collenchyma cells are present in the ridge of epidermis. The pith is present at the centre, which is surrounded by vascular bundle and xylem vessels (Figure 7).

Root: T.S. of the root shows circular margin with minute trichomes. The outer most layers is epidermis followed by dense layer of cortex. The cortex which consist of xylem and phloem. Xylem is endarch, where protoxylem faces towards the centre and metaxylem faces towards the periphery. The xylem surrounds the phloem. The medullary rays are present between xylem and phloem. The xylem vessels are dense and large in size (Figure 8).

Petiole: C.S. of the petiole is rhomboidal shaped. The abaxial side is U shaped. The outer layer is epidermis which consists of minute trichomes. The vascular bundles are 6 in number, which is surrounded by pith. The vascular bundle consists of xylem and phloem. The xylem is exarch in condition. The pith is at center with cluster of cells (Figure 6).

According to the morpho/anatomical observation of both study species was found with most similar characters as consider being a closely related species. It is clearly indicates the evolutionary relationship between the species. In overall results the study species *Cardiospermum halicacabum* was morphologically differentiated with *C. corindum* by leaf, stem, internode and fruit characters. It is observed that stalked main leaflets, five angled stem, 4-7 cm internodal length, sharp leaf tip without mucronation, and small triangular ends on fruit in *C. halicacabum*; and sessile main leaflets, six angled stem, 8 cm long internode, sharp leaf tip with mucronation, large balloon shaped fruit in *C. corindum*. Also *Cardiospermum halicacabum* has non-inflated capsules that differ from the inflated capsules of *C. corindum*. This study was agreed with Stella and Maria, 2006, they found *Cardiospermum procumbens* and *C. pterocarpum* are closely related; they are basically distinguished by the leaf type, simple in the first species and compound in the second one; in addition to the characteristic domatium of *C. procumbens*. These species share the same habit, foliar structure and epidermal characters.

In anatomical features of these two species were differentiated with some prominent characters. Especially for *Cardiospermum halicacabum* the presence of anamocytic type of stomata with diffused in number, stem pentagonal, having 5 short blunt ridges and the xylem vessels are diffused and small in size in root. Whereas in *C. corindum* it was observed dense anamocytic type of stomata, hexagonal stem having 6 short ridges and the xylem vessels in root are dense and large in size. Based on this study the stomatal number variation is one of the environment dependant (Engineer *et al.*, 2014). Previous studies have shown that

stomatal density is negatively correlated to stomatal size or length (Franks & Beerling, 2006) and that stomatal characteristics are susceptible to environmental changes, such as light intensity, temperature, and water status. These evidence are identified additional characters for differentiate the study species properly.

CONCLUSIONS

The morpho/anatomical studies provide more additional characters for proper identification of plant species. The morphological characters of leaf size, shape and pattern, hairiness, petiole nature, stem features, inflorescence, fruit and seeds; the anatomical characters like stomatal characters, epidermal layers, mesophyll cells, vascular bundles arrangement, etc. are mostly used to differentiate for the selected study species. This could be the additional taxonomic evidence than the available floras descriptions. It may help to proper identification of closely related allied plants and also it is an important tool in studying evolutionary history and ecological relationships between the species.

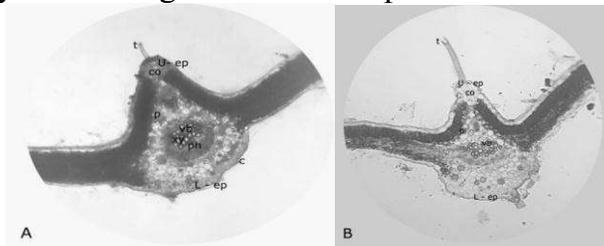


Figure 5. Cross section of the leaves. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (U-ep: Upper epidermis, L-ep: Lower epidermis, c: Cuticle, co: collenchyma, p: parenchyma, vb: vascular bundle, xy: xylem, ph: phloem, t: trichome.

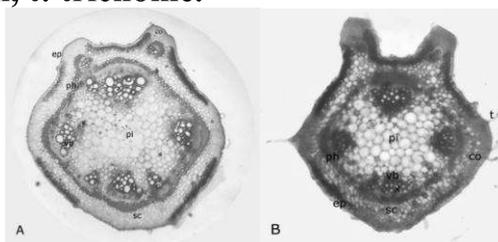


Figure 6. Anatomical characters of the petiole. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (ep: epidermis, co: collenchyma, sc: sclerenchyma, vb: vascular bundle, xy: xylem, ph: phloem, t: trichome, pi: pith.

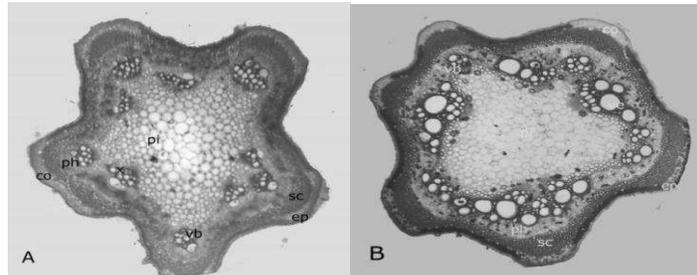


Figure 7. Cross section of stem. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (ep: epidermis, co: collenchyma, sc: sclerenchyma, vb: vascular bundle, xy: xylem, ph: phloem, pi: pith).

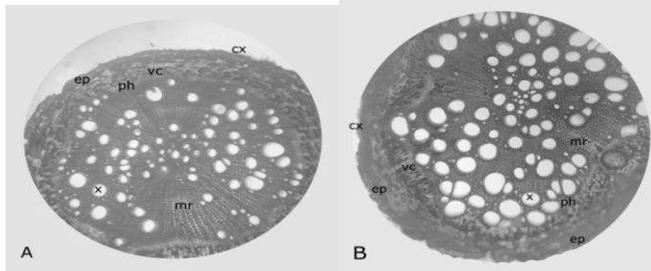


Figure 8. Cross section of root. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (ep: epidermis, cx: cortex, vc: vascular cambium, x: xylem, ph: phloem, mr: medullary rays).

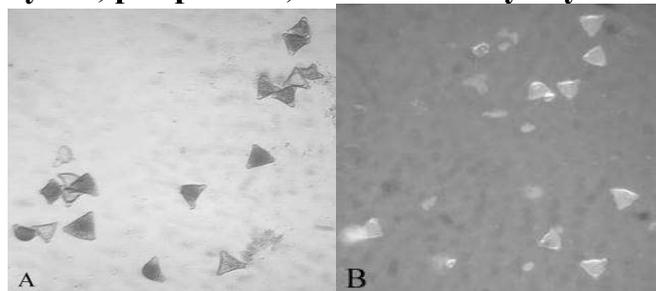


Figure 9. Morphological structure of Pollen. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L.

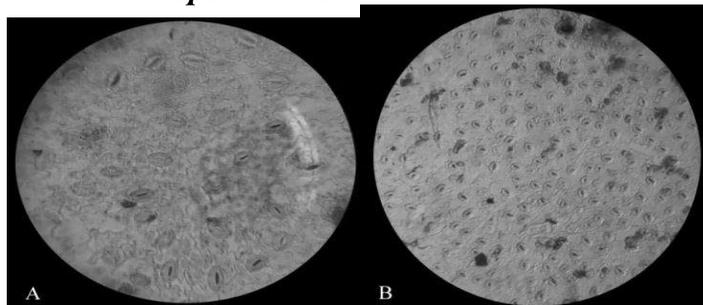


Figure 10. Epidermal peeling of leaf shows stomatal structures A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L.

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REVIEW OF ENVIRONMENTALLY FRIENDLY COPPER AND COPPER OXIDE-BASED NANOPARTICLES AND THEIR USE IN ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Utilising plant extracts as reducing and capping agents, this critical review focuses on the state of green synthesis of copper and copper oxide (Cu/CuO)-based nanomaterials and examines the antibacterial uses of these biomaterials. Additionally, a brand-new speculative mechanism for the antimicrobial activity of Cu/CuO nanomaterial produced through biological means was put forth. The future of environmentally friendly Cu/CuO nanomaterial production for antimicrobials will be clarified by this study. Researchers are able to quickly understand the synthesis process using plant extracts by reading this review's explanation of the synthesis approach and the plant components that have been previously applied. This publication also provides a synopsis of the microbial strains employed in this arena and an overview of the various analytical techniques employed for characterising the generated nanomaterials.

Introduction

The study of matter at the nanoscale (1-100 nm) is the interesting and fast expanding field of research known as nanoscience. When compared to their bulk counterparts, the nanomaterials display unique characteristics. Due to their special characteristics, including a high surface-to-volume ratio, form, size, and composition, nanomaterials have a tremendous potential for a variety of applications [1]. In recent years, metal and metal oxide nanoparticles have significantly improved biomedical sensing, imaging, diagnosis, and treatment. The three metals which are utilised the most regularly today are silver, copper, and gold. A low-cost, high-yielding material that can be exploited in biomedical and ecological restoration applications among these metal nanoparticles is copper

[2]. Due to their intriguing properties, which make them useful in a wide range of fields, such as solar cells [3], environmental remediation [4,5], catalysts [6, 7], sensors [8, 9], optics [10], electronics [11], antimicrobials [12,13], etc., copper and copper oxides-based nanomaterials have attracted a lot of attention in recent years. There are several other mechanisms that can be used to create these nanostructures, including physical, chemical, and biological ones. Even though physical and chemical processes generate a large number of nanoparticles, they are not preferred due to the use of hazardous materials, high costs, and energy requirements. Green methodologies were developed to address the limitations of physical and chemical processes. The green procedure for synthesis incorporates resources that are both organic and natural. Many viruses, bacteria, and fungi are evolving resistance to the antimicrobials that have become available. New microbial species are also developing for unknown reasons. Microbiological diseases had become a serious threat to humanity. This may be due to the ineffective use of current antimicrobial medications or unsustainable development that is bad for the environment and ecology. The discovery of novel antibiotics is essential in the event of pandemic infections [14].

In this review, which covers the literature up to 2021, we summarise and elaborate on green synthesis using plant extracts and their usage as antimicrobial agents. This article provides an overview of the methods for creating Cu/CuO-based nanomaterials, as well as their production mechanism, optimisation parameters, and characterisation. The use of Cu/CuO nanoparticles as antibacterial agents is the paper's main topic. In order to contribute to the growth of the literature on this topic and to support researchers in their future endeavours, the final goal of this paper is to reveal the procedures and results of the green synthesis of Cu/CuO nanomaterials. The emergence of new pandemic diseases is crucial.

Green synthesis of Cu/CuO based nanomaterials

The manufacturing of nanoparticles uses plants with antioxidant characteristics. Precursor salts can only be reduced by such plant parts since they have reducing characteristics. The majority of therapeutic plants contain antioxidant qualities. According to reports, the biomolecules contained in plant extracts, such as proteins, phenols, flavonoids, carboxylic acids, tannins, terpenoids, etc., are what reduce and cap the nanoparticles [15-17]. An analytical-grade precursor copper salt solutions, such as copper acetate, copper sulphate, copper nitrate, copper chloride, etc., and different parts of the plants were used. Plant parts that are fresh or dried and powdered are dissolved in distilled water.

Plant parts that are fresh or dried and powdered are dissolved in distilled water. Most frequently, distilled water is employed as the solvent. Now, depending on the volatile nature of the phytochemicals present in the plants, it is boiled at various temperatures, which can be achieved through a variety of extraction techniques. The extract is now centrifuged and removed using Whatmann No. 1 filter paper and muslin cloth. A certain amount of the filtered extract is measured out and combined with the particular quantity and molarity of the precursor solution [15]. The formation of nanoparticles can be detected using a UV-Visible spectrum, causing a change in colour.

Effect of temperature on green synthesis of Cu/CuO based nanomaterials

Temperature plays a key role in the biosynthesis of Cu/CuO-based nanomaterials. Ideal temperatures are below 100 °C. Typically, ambient temperature is maintained for synthesis. Inadequate for the formation of nanomaterials are temperatures above 70 °C. This is a result of plant phytochemicals being extremely volatile. Furthermore, it has been demonstrated that temperature has a significant influence on the shape and size of nanoparticles. Nagar et al. found that the conversion rate increased with reaction temperature when producing copper nanoparticles. The optimal temperature for this investigation was 85 °C [18]. Similarly, Dlugosz et al. showed in 2020 that the size of the nanoparticles decreases as the temperature increases when water is used as the solvent.

Additionally, they discovered that all systems of metal-based nanoparticles shrank in size at 60 °C, irrespective of the precursor salt [19]. Additionally, they demonstrated that the creation of nanoparticles accelerates with rising temperature. Additionally, at extremely high temperatures, the leaf extract's phytochemical constituents lose their ability to stabilise, resulting in the loss of their stabilising properties. Similar results were obtained when copper nanoparticles were produced by *Piper retrofractum* fruits. The peak absorption strength in this case was discovered between 60 and 80 °C [20].

Effect of plant extract concentration on green synthesis of Cu/CuO based nanomaterials

The kind and components of plants utilised are fully necessary for the biosynthesis of Cu/CuO-based nanomaterials. Different plant species phytochemicals have varying impacts on the synthesis, capping, and biological activity of nanomaterials. The ability of the copper salt solution to reduce to

copper or copper oxide nanoparticles is determined by the reducing power of phytochemicals in the plant extract, which plays a crucial role in the reduction mechanism. Furthermore, depending on the plant species used, the composition of the nanomaterials produced will vary. According to earlier studies, the synthesis process is speed up by adding more plant extracts. Insufficient absorption was seen, indicating insufficient copper ion reduction, when a little volume of leaf extract (5%) was used to create copper nanoparticles [18].

Effect of pH on green synthesis of Cu/CuO based nanomaterials

The pH can be altered by adding basic or acidic solutions to the reaction media. Sarwar et al. added citric acid to the reaction mixture to change the pH [21]. Citric acid was added, which helped the reduction process over time. To regulate the size and shape of nanomaterials, the pH of the medium is also altered. Additionally, pH significantly affects capping and stabilising properties, and consequently, the development of nanoparticles [22]. In 2020, Amaliah et al. presented a thorough analysis of the size of nanoparticles at various pH levels. They found that compared to alkaline (pH 10) conditions, acidic (pH 4) conditions produced more uniform dispersion and smaller nanoparticles [20]. In acidic conditions, some phytochemicals may lose their activity.

Application of Cu/CuO nanomaterials as antibacterial agents

Testing for action against diverse bacteria, viruses, and fungi is a part of antimicrobial research. In the study of antimicrobial substances, both gram-positive and gram-negative microbes have been studied. It has been established that the biomolecules involved in the production and capping processes affect the antibacterial activity of plant extracts. Even under identical biosynthetic conditions, proteins functionalized Cu/CuO based nanomaterials show varying biological activity when produced with various plant extracts. [14,15].

Biofunctionalized nanoparticles have shown some antibacterial activity, however the exact mechanism continues to be unknown. Researchers have put forth several potential antimicrobial action mechanisms, including as oxidative stress injury, mechanical damage, and gene toxicity. It is suggested that another mechanism underlies the antibacterial activity of biosynthesized Cu/CuO nanoparticles. Microbial adsorption on the surface of biofunctionalized nanomaterials, which is predominantly caused via chemisorption, may be responsible for the antibacterial effect. This occurs brought on by non-electrostatic interactions between the nanomaterials and the microbial surface,

which include the Vandervaals force, hydrogen bonds, and others. In a study, it was found that a Cu/CuO based nanomaterial made from *F. religiosa* possesses both adsorption and antibacterial properties [15].

Conclusion

This study sheds light on how plant extracts can be used to biosynthesize Cu/CuO-based nanomaterials, which are then used in a wide range of antibacterial applications. A brief description of the general synthesis strategy and characterisation techniques is given. There is a full discussion of the suggested processes for synthesis and antibacterial application. A unique mechanism has been connected to the antibacterial action of green nanomaterials, including plant extracts. There has been a large body of research on the antibacterial effects of biosynthesized Cu/CuO-based nanomaterials.

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