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 Mathiew 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 tree lets, exceptionally sub-shrubs or scandent . Vast majority of Sapin-Daceae genera have trilocular fruits, although many also have bilocular, unilocular, or sometimes quadrilocular fruits. (Sapindaceae Juss. (1789), *Aceracea* 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 thyrse, 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 ± 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, dithecous, 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).

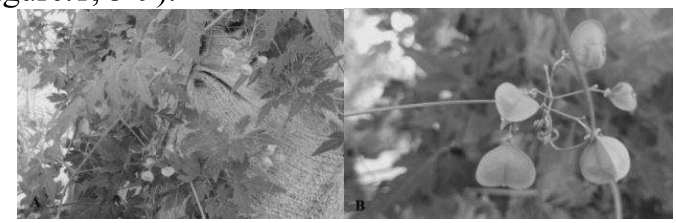

Figure 1. **A. Habit of *Cardiospermum halicacabum* L. B. Twig with fruits**

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 thyrse; 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, dehiscing 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, triporate (Fig 2, 4-9).

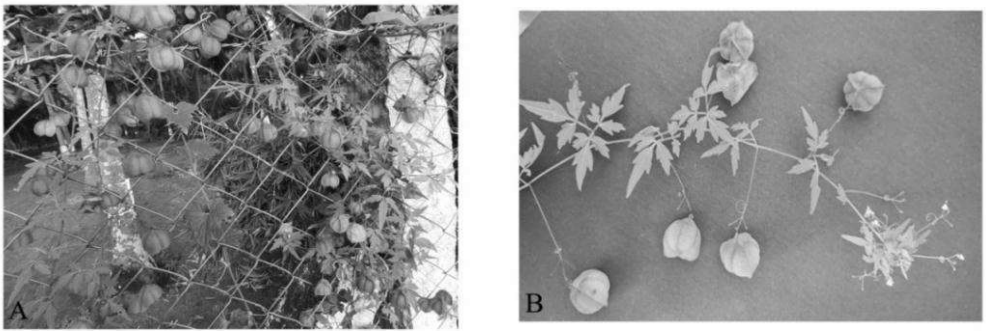

Figure 2. **A. Habit of *Cardiospermum corindum* L., B. Twig with fruits**

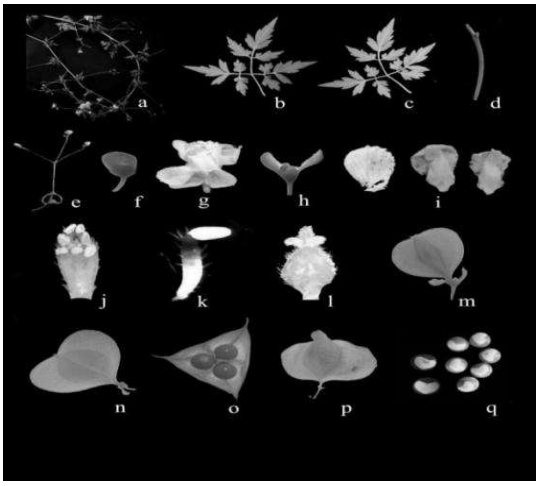

Figure 3. Morphological structures of *Cardiospermum halicacabum* L. – a) Habit, b) Adaxial leaf, c) Abaxial leaf, d) Stem, e) Inflorescence, f) Flower Bud, g) Flower, h) Calyx, i) Corolla, j) Androecium, k) Stamen, l) Gynoecium, m) Young fruit, n) Mature fruit, o) C.S of ovary, p) Fully matured fruit,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

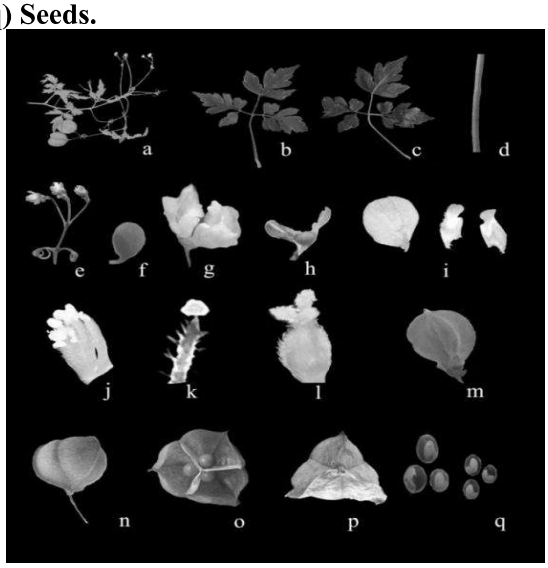

Figure 4. Morphological structures *Cardiospermum corindum* L. – a) Habit, b) Adaxial leaf, c) Abaxial leaf, d) Stem, e) Inflorescence, f) Flower Bud, g) Flower, h) Calyx, i) Corolla, j) Androecium, k) Stamen, l) Gynoecium, m) Young fruit, n) Mature fruit, o) C.S of ovary, p) Fully matured fruit, q)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

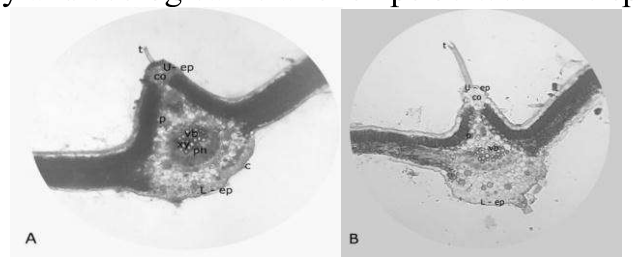

Figure 5. Cross section of the leaves. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (U-ep: Upper epidermis, L-ep: Lower epidermis, c: Cuticle, co: collenchyma, p: parenchyma, vb: vascular bundle, xy: xylem, ph: phloem, t: trichome.

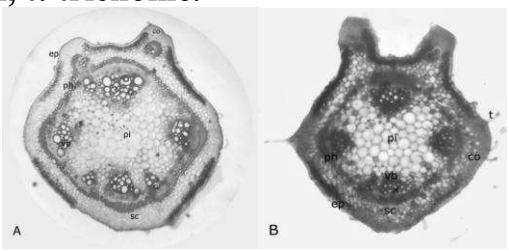

Figure 6. Anatomical characters of the petiole. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (ep: epidermis, co: collenchyma, sc: sclerenchyma, vb: vascular bundle, xy: xylem, ph: phloem, t: trichome, pi: pith.

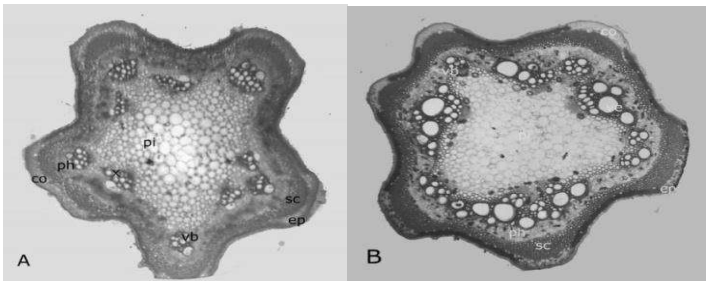

Figure 7. Cross section of stem. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (ep: epidermis, co: collenchyma, sc: sclerenchyma, vb: vascular bundle, xy: xylem, ph: phloem, pi: pith.

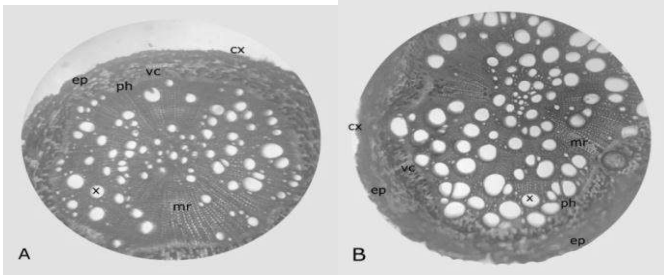

Figure 8. Cross section of root. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L. (ep: epidermis, cx: cortex, vc: vascular cambium, x: xylem, ph: phloem, mr: medullary rays.

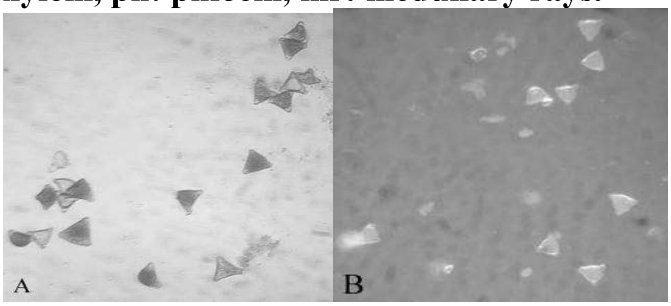

Figure 9. Morphological structure of Pollen. A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L.

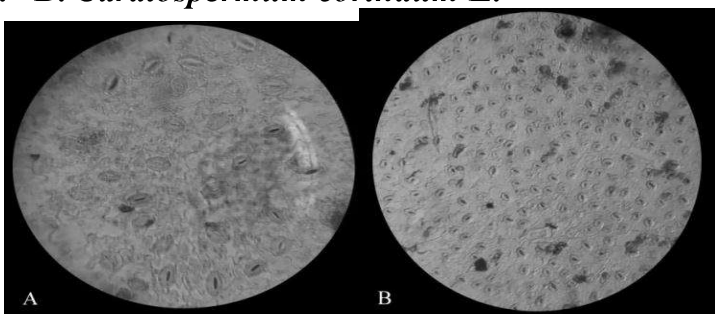

Figure 10. Epidermal peeling of leaf shows stomatal structures A. *Cardiospermum halicacabum* L. B. *Cardiospermum corindum* L.

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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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