

Metabolomic Profiling, Industrial and Commercial Prospectives of *C. procera*

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Abstract: *Calotropis procera* (Aiton) Dryand, sometimes referred to as the apple of Sodom, giant milkweed or calotrope, is a perennial shrub that belongs to the Apocynaceae family. It is commonly called "Ushar" or "Oshar" in Arabic. *C. procera* is predominantly found in places that are semi-arid and arid. Ushar is a xerophytic perennial shrub native to the Arabian Peninsula, Africa, Western Asia, the Indian Subcontinent, and China. It has been introduced to Central and South America, Australia, and the Caribbean islands. It is a versatile plant that can be used for phytoremediation, the synthesis of nanoparticles, fiber production, medicine, feed, and fuel. Several facets of *C. procera* have been covered in this review, including its general traits and present and future applications, especially in the industrial and commercial domains. Besides that, the major compounds identified in the literature survey of the different parts of *C. procera* extracts, they found to have a variety of bioactivities. This review's goals are to: a) gather data from the literature on *C. procera* and make it easily readable for further study; b) investigate the metabolic profile of the flowers, leaves, stem, roots, root bark and latex of *C. procera* that could be used in different aspects with pharmacological potential, and c) highlight the gaps in the knowledge of *C. procera* industrial properties in some local areas in Indian and West African countries and economic characteristics like rubber production besides its application in agriculture to feed, pesticides, bio-fungicides, biostimulants, and bionutritive, in addition to, its role in green nanotechnology.

Key words: *Calotropis procera* (Aiton) W. T. Aiton • Metabolomic Profiling • Industrial Properties

INTRODUCTION

Calotropis procera (Aiton) Dryand (*C. procera*) is a perennial, softwood shrub. It belongs to the subfamily Asclepiadaceae, which is characterized by a milky latex, and is a member of the Apocynaceae family [1-3]. "Calotropis" is derived from the Greek word for "beautiful," which refers to its flowers; while "procera" is a Latin term for the cuticular wax present on the plant's leaves and stems [4].

The most prevalent synonym for the species is *Asclepias procera* Aiton [5]. *C. procera*, it is known by a variety of common names, including 'Ushar' or 'Oshar' in Arabic [4, 6]. Besides, it is known in many regions of the

globe as Sodom apple, giant milkweed, Madar, rubber tree, wild cotton and Calotrope cabbage tree [4, 7]. *C. procera* is an evergreen perennial shrub with a deep taproot system, reaching 3-4 m deep Figure 1 [7]. Its conspicuous, rounded apexes produce large tubers, while the rest is spirally curved [8,9]. The roots are whitish-grey and exude sap when the bark is severed. The cork's exterior layer is spongy and rough, while the interior layer is smooth and mucilaginous [4].

C. procera stems are soft-wooded shrubs, 2-6 m tall, with a 25-cm diameter [9]. The crown diameter can reach 6.9 m. Young stems are grayish-green, silky, and covered with white hairs, while mature stems have a fissured, light brown bark resembling cork Figure 1-B [4, 7, 9].

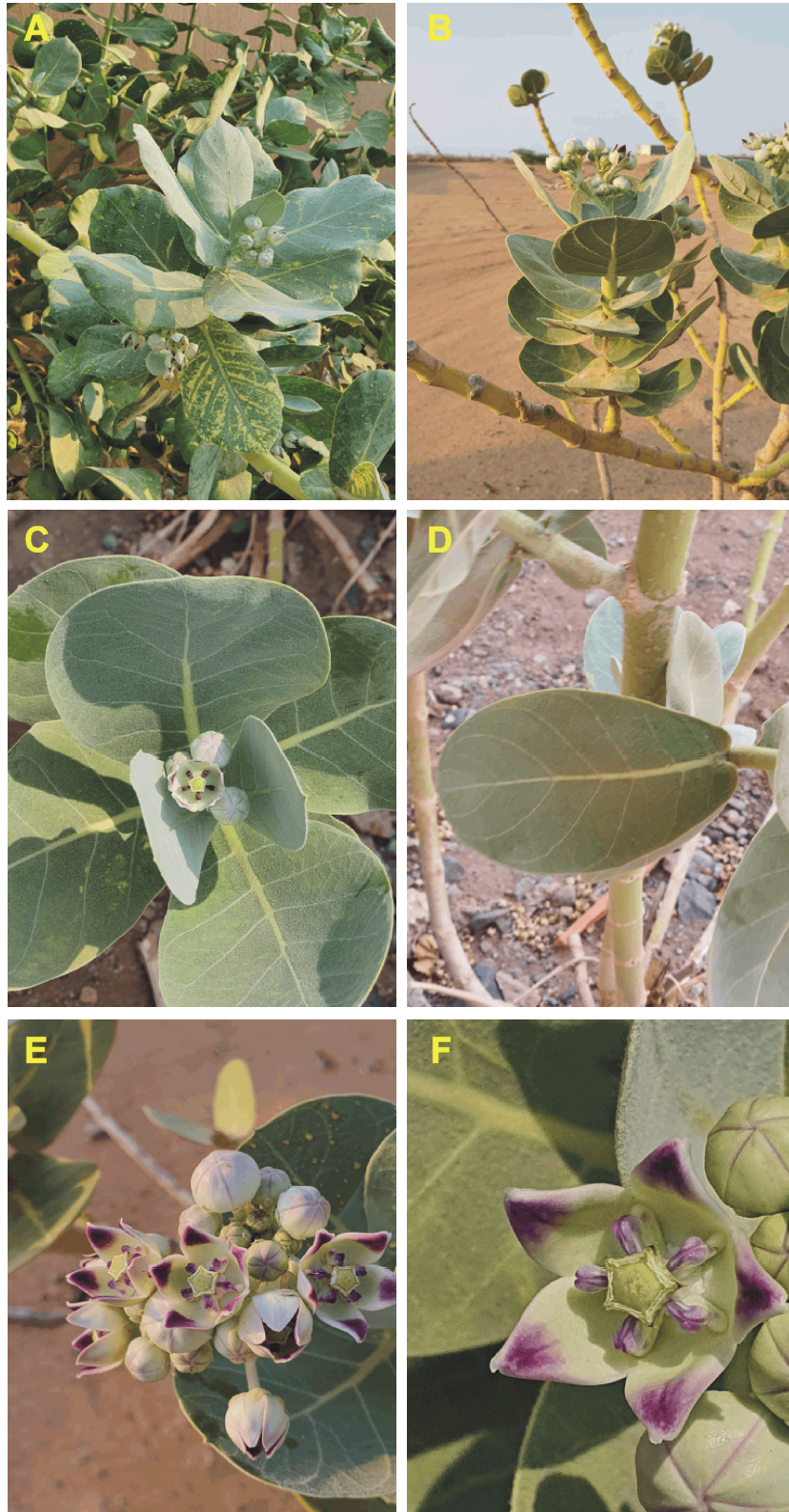


Fig. 1: The *C. procera*: flowering plant (A&B); phyllotaxy (C); a young leaf (D); inflorescence and reproductive buds (E); individual flower (F).

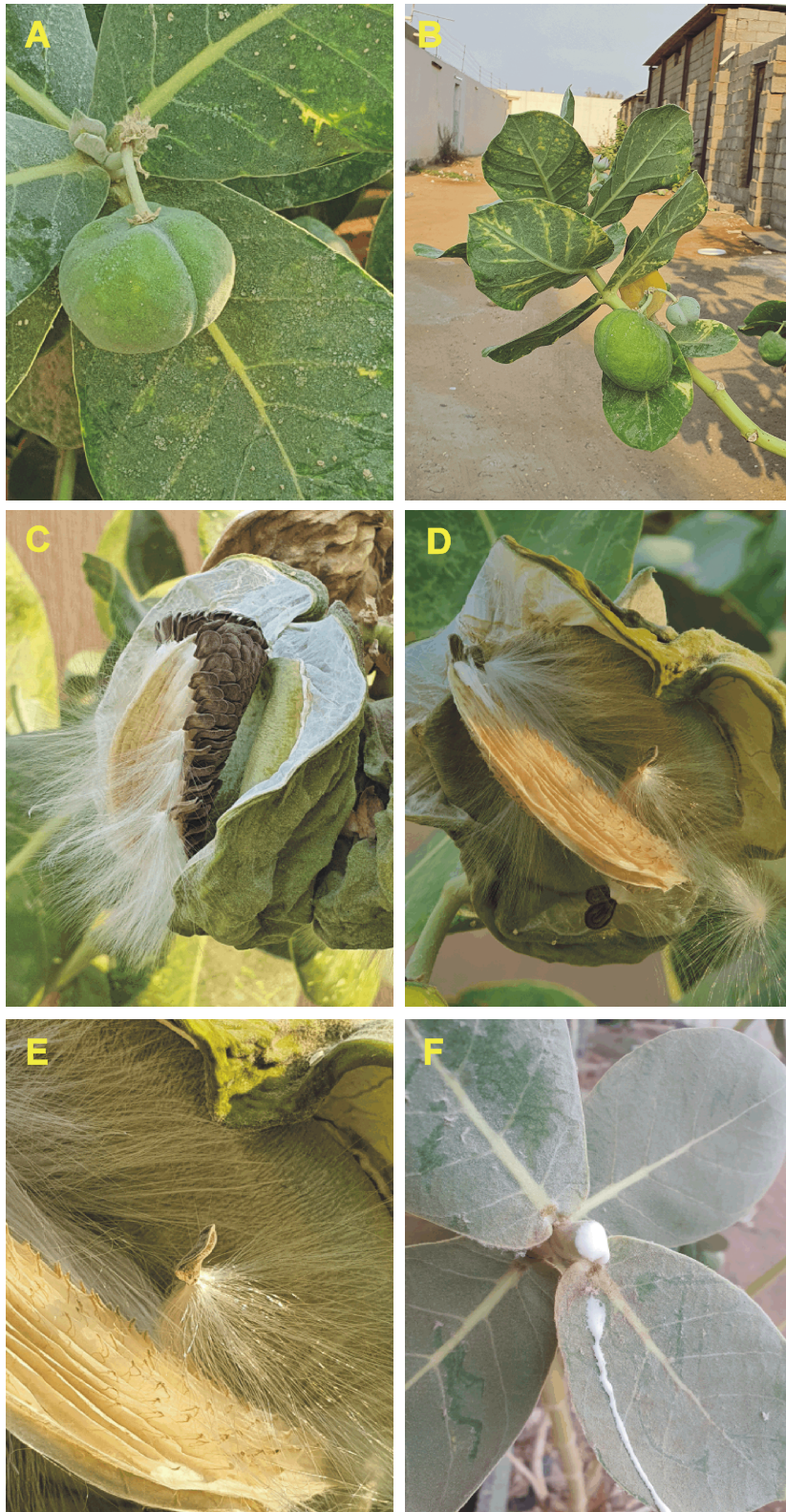


Fig. 2: Fruit characteristics of *C. procera*: immature fruits (A); very small immature and mature fruits (B); Dehiscence fruits and the seeds released and dispersed by wind (C & D); An magnifying image of the seeds with silky white pappus (E); and latex leaking out from the broken stem (F).

acetate A, calotropoceryl acetate B, calotropoceronone A, calotropocsterol A, taraxasterol, pseudo-taraxasterol acetate, (*E*)-octadec-7-enoic acid and calotropursenyl acetate B, besides one steroid stigmasterol compound from the root bark of this plant. These compounds were found to have growth inhibitory activity in three human cancer cell lines *in vitro*. *C. procera* latex containing high concentrations of biologically active chemicals such as alkaloids, saponin, steroids, flavonoids, triterpenoids, tannins, triterpenes, caoutchouc, calotropin, cardenolides, gallic acid, quercetin, glycosides, laticifer protein osmotin, procerain, procerain B, and two different cysteine peptidases [34-38]. Three of the new proteins described by Ramos *et al.* [37] process identical sequences of N-terminal amino acid and in terms of enzymatic activity, these enzymes showed distinct variations across a wide variety of pH and temperature conditions. In this vein, two new proteins were identified by Freitas *et al.* [39] in the usher latex and categorized as germin-like proteins (GLPs) called *C. procera* germin-like proteins (CpGLP1 and CpGLP2), which play a key role in plant defense. The two proteins were found in the latex but not in the seeds germinated or non-germinated and calli. These results point out that the latex components of *C. procera* are tissue-specific. The other evident refer to that is the latex in the stem and roots is more cytotoxic compared to that in leaves, as they composed of calotoxin, calotropin and calactin, while the latex on leaves consists of glycosides calotropin, uscharin, as well as calotoxin [38]. On the other hand, there are biochemically active compounds are existed in the whole ushar plant, such as steroids, terpenes, flavonoids, and cardenolides [40]. Moreover, Bader *et al.* [41] found that the cardenolides appear in all part of *C. procera* including the latex, however, cardenolides were notably abundant in latex and leaves involving uscharidin, calactin, 15 β -hydroxy-calactin, 16 β -hydroxy-calactin, and 12 β -hydroxy-calactin.

Industrial and Commercial Prospective:

Using *C. procera* as Fodder: One of the economic benefits of this plant is that it is used for feeding animals in times of scarcity, *i.e.*, goats, camels, and sheep can be fed on young pods, senescing leaves, and flowers [42]. Additionally, as a few previous studies suggested using the aerial part of *C. procera* in animal feeding, these recommendations result from nutritional aspects found by Vaz *et al.* [43] and Abbas *et al.* [44] by studying the chemical composition of *C. procera*, they discovered that dry matter percent found to be ranged from 88.7 to 94.6%,

crude protein from 19.4 to 21.2%, neutral detergent fiber from 19.5 to 29.5% and acid detergent fiber from 19.6 to 21.0%. They also suggested feeding it to tiny ruminants at a rate of up to 500 g/day as a 50% blend with hay from other forages. It has been suggested that the semi-arid regions of Brazil and India are suited for using silk flower hay as a feed for small ruminant animals, such as sheep, goats, and camels [45, 46].

Several studies examined the use of silk flower hay, and the results showed no suppressive effects on the meat quality of animals. There is a considerable attempt to detect the accurate amount of *C. procera* silk flower hay. According to Marques *et al.* [47], Santa Inês sheep can have up to 33% of their forage sorghum hay replaced in their diet without any negative effects on their performance or the quality of their carcasses. Moreover, in order to assess potential impacts on the growth and quality of the meat of the male Santa Inez lambs, Madruga *et al.* [46] studied the different levels of silk flower hay of *C. procera* in the diet of confined lambs in place of the often utilized forage broom corn hay (*Sorghum bicolor* L.). The amount of forage substituted reached up to 50%; however, the overall quality of the meat was unaffected. In this experiment, different amounts (16.7%, 33.3%, and 50%) of silk flower hay in the diet were tried. Using 16.7% was found to be the most appealing and technically possible choice for the Northeast region of Brazil. Torres *et al.* [48] concluded that the performance and nutrient consumption of Morada Nova sheep could be unaffected by replacing up to 30% of the corn and soybeans in the concentrate with silk flower hay. Furthermore, the quality of the meat and carcass, leg weight, tissue composition, hydration, juiciness, and flavor were investigated by the usage of silk flower hay in the diet. Costa *et al.* [45] found that feeding Morada Nova lambs silk flower hay instead of corn (26.67%) and soybean (3.33%) had no effect on the tissue composition, ratios, muscularity index of the leg, and physical-chemical properties of the semimembranosus muscle.

However, the latex contains poisonous elements that can be hazardous to livestock [49]. *C. procera's* acute toxicity was examined by Mahmoud *et al.* [50] in Desert sheep and Nubian goats when latex from the plant was administered orally, intravenously, intraperitoneally, or subcutaneously at doses ranging from 6 to 0.001 ml per kg. Both species died when *C. procera* latex was administered to them via any method. The animals exhibited anxious symptoms, increased urination, tachycardia, dyspnea, and condition loss. The liver, kidneys, heart, lungs, brain, and intestines all displayed

severe pathological alterations. Changes in the serum concentrations of total protein, ammonia, bilirubin, sodium, potassium, calcium, and creatinine were linked with these. The total lipid levels in the kidneys, heart, and liver increased. Additionally, a few research reporting harmful effects induced by *C. procera* latex support these conclusions [51-53]. Also the similar harmful effect of fresh *C. procera* leaves ingestion induces acute cardiotoxicity and hepatotoxicity in sheep have reported by Mahmoud *et al.* [50] De Lima *et al.* [51].

Using *C. procera* as Biofuel: The *C. procera* plant is one of a few plants that can be used as biofuel. Its traits are crucial for the production of biomass, which is used to create bioenergy. Moreover, being crucial for producing liquid biomass, such as non-edible oil and hydrocarbons, in addition to solid biomass, providing an alternate source of petroleum. Due to their capacity for water conversion, they can survive on a limited amount of water and still produce an adequate amount of biomass. As well as mostly undesired by the cattle folk because of toxic latex [38, 54, 55]. According to Behera *et al.* [56] the dried biomass of *C. procera* yields 3.8% of dry matter from the stem and 5.1% from the leaves in hexane extract. While methanol extract production from the stem and leaves of the dried biomass of *C. procera* is estimated to be 18.5% and 12.2% dry matter, respectively. In parallel studies, methanolic extraction produced the best results; the hydrocarbon content in the *C. procera* plant was 38.8% when extracted using the solvent methanol and 6.2% in hexane extract. Moreover, for extracting non-edible oil, dried seeds were collected and used, yielding 36.2%. Further, the study suggests plant biomass can be transformed into liquid fuels [54]. The same advocated was provided by Kumar [47], who found that *C. procera* latex has 30% hydrocarbons, primarily triterpenoids, and that these hydrocarbons are capable of being converted to biofuels. Therefore, this plant has the potential to serve as a biofuel source in semi-arid and arid regions of the globe.

In a recent study, sequential pretreatment methods were applied to the biomass of *C. procera* fruits and leaves. An organic solvent (hexane/methanol) was used in a two-step pretreatment process to separate biocrude and plant wasted residues, then spent residues of fruits (SRF) and leaves (SRL) were obtained from the plant waste by subjecting the waste fractions to acid/alkali (1% H₂SO₄/2% NaOH) pretreatment in succession. Both the SRF and SRL that were sequentially pretreated had high levels of cellulose (87.3% and 83.4%,

respectively). After being subjected to Sternzym cellulase hydrolysis, 10 g of sequentially pretreated SRF and SRL yielded 80.2 and 50.4 g/L of total reduced sugars, respectively. Following the sugars were reduced, *Saccharomyces cerevisiae* was used to ferment them into bioethanol. The High-performance liquid chromatography results indicated that sequentially pretreated SRF and SRL produced 38.9 and 23.8 g/L of bioethanol, respectively. In this work, researchers proposed using *C. procera*, a cheap, non-edible plant biomass, as a biorefinery to create sustainable bioethanol [58]. The *C. procera* can be a biogas potential source, the first attempt to investigate that by Djimtoingar *et al.* [59]. Positive findings from the experiments showed that the plant stem generated biogas at a rate of 17,744 mL and biomethane at a rate of 1.439 L/g.VS, while the leaves generated biogas at a rate of 8500 mL and biomethane at a rate of 0.4409 L/ g.VS.

The Bioactivity of *C. procera* in Agriculture:

Bio-Pesticide Activities of *C. procera*: The pesticide bioactivity of *C. procera* were reported in several studies, Bakavathiappan *et al.* [60] investigate the effect of its leaf extract on third instar larvae of *Spodoptera litura* to determine the antifeedant activity of various solvent extracts. The chloroform extract showed the highest level of activity, followed by hexane, ethanol, acetone, ethyl acetate, and methanol. Extract of chloroform was the most effective extract as larvacide against *S. litura* and the concentration of the extract was directly proportional to the antifeedant activity. The results demonstrate that the chloroform extract of *C. procera* possesses numerous insect-controlling properties. In other study Esmaily *et al.* [61] used the extract of *C. procera* on the *Bemisia tabaci* parasite and compared the results to those obtained by exposing the parasite to four synthetic pesticides: pymetrozin, imidacloprid, diazinon, and abamectin. The results of this study imply on the potential of *C. procera* extract for controlling *B. tabaci* growth compared to chemical pesticides. Furthermore, the *C. procera* was considered as a green pesticide, the ovicidal activity of plant organ extracts (flowers, foliage, branches and roots) and latex against *Cadra cautella* was evaluated. *C. procera* root extracts were the most efficacious, preventing 50% of *C. cautella* embryos from hatching at 10,000 ppm (1%) [41].

Bio-Fungicides Activities of *C. procera*: The other role of *C. procera* bioactivity is fungicides. The activity of the peptide fraction from *C. procera* (PepCp), which was

isolated from the latex, was studied against the fungus *Colletotrichum gloeosporioides*, and the results established that the mycelial growth of the fungi was inhibited by 80% [62]. Moreover, Abo-Elyousr *et al.* [63] utilized the aqueous extract of *C. procera* to investigate its efficacy against *Fusarium* wilt disease (Fwd) of tomatoes, which is caused by *Fusarium oxysporum* f. sp. *lycopersici*. The results showed that all *C. procera* extract concentrations inhibited the growth of the pathogen. The aqueous extract at a concentration of 15% achieved the highest decrease in mycelia growth, bringing the pathogen's growth down to 70.2% of the control level. Furthermore, putative antimicrobial α -helical propeptides (SnuCalCpIs) derived from *C. procera* was found to inhibit the activity of four food-spoiling yeasts: *Candida albicans*, *Saccharomyces cerevisiae*, *Pichia anomala*, and *Rhodotorula mucilaginosa*. SnuCalCpI15 initially binds to the surface of yeast cells and then enters the cells via an increase in cell membrane permeability; hence, yeast cells undergo morphological alterations, most notably a change in cell wall thickness. According to the findings, SnuCalCpI15 is a potent alternative to chemical antifungal food preservatives and can be used to increase the shelf life and quality of foods susceptible to yeast spoilage [64].

Using *C. procera* as a Bio-Stimulant, Bio Nutritive and Herbicide: A limited number of researches studied the stimulatory and nutritive effect of *C. procera* extract on different plant species. Abeed *et al.* [65] used leaves extract of *C. procera* as a bio-stimulant for the growth and quality of *Catharanthus roseus* as determined by a number of physio-biochemical indices. The stimulation effects appeared on *C. roseus* plant after the treatment with the *C. procera* extract by foliar spraying or irrigation. The length of roots and shoots, as well as their dry and fresh weight, were significantly enhanced. Beside the significantly increased abundance of bioactive molecules in *C. roseus*, including anthocyanins, phenolics, flavonoids, alkaloids, ascorbic acid, reduced glutathione, and tocopherol. Despite the use of polluting fertilizers, the application of CLEs is a promising strategy for enhancing the yield and quality of plants, according to the findings of the study. The allelopathy phenomenon was also investigated in *C. procera*. The effects of different concentrations (0.8%, 0.1%, and 1.2%) of *C. procera* leaf extracts on the germination and growth of maize seedlings were studied in the laboratory and in pots. The aqueous leaf extracts of *C. procera* were found to improve the germination. In addition, all

concentrations of *C. procera* extracts stimulated the growth of maize seedlings (root length, shoot length, and fresh and dried weight of root and shoot). This study indicated that *Zea mays* could be planted near these naturally occurring plant species due to their growth-stimulating properties [66].

On the other hand, Al-Zahrani and Al-Robai [67] investigated the impact of *C. procera* dried leaf water extract at five concentrations (5, 10, 20, 40, and 60%) on the germination of *Triticum aestivum*, *Cucumis sativus*, *Senna occidentalis*, *Hordeum vulgare* and *Trigonella foenum-graecum*. The experimental results demonstrated that increasing leaf extract concentration slowed germination and decreased the ultimate germination percentage. However, the lower concentration (5%) stimulated plumule emergence and growth more than the control treatment in *Trigonella foenum-graecum* and *Senna occidentalis*, then length of the plumule decreased as the concentration of leaf extract from *C. procera* increased. This negative effect on the germination represents a bioactivity of *C. procera* as an herbicide, grass *Ischaemum afrum* seeds were treated with different concentrations of aqueous extracts from leaves, inflorescences, stems and roots of *C. procera*. The results showed that the extracts from the different parts suppressed seed germination of *I. afrum* and the most toxic extraction was from leaves, followed by inflorescences followed by roots. Additionally, there was a positive relationship between the extract concentration and inhibition percentage of seeds [68]. Likewise, the extraction of aerial parts of this plant has inhibited seed growth of *Chenopodium murale* and *Portulaca oleracea*, undesirable plants [69].

Industrial Application:

Rubber Industries: Previous researches demonstrates that *C. procera* is rich in all components that can be utilized in different industrial applications (Fig. 3). Ramos *et al.* [70] estimate that 84% and 9% of the dried matter of *C. procera* latex is composed of rubber fraction and soluble proteins, respectively. The natural rubber consists of the natural polymer, namely poly(cis-1,4-isoprene), therefore the latex involved in rubber industrial products such as gloves, tires, balls and balloons [71, 72].

Cheese Making: *C. procera* extracts are used as milk coagulants in cheese making. In West African countries such as Nigeria the extract of this plant is traditionally used in cheese production [73]. Furthermore, Freitas *et al.* [74] found that a *C. procera* latex containing a mixture of

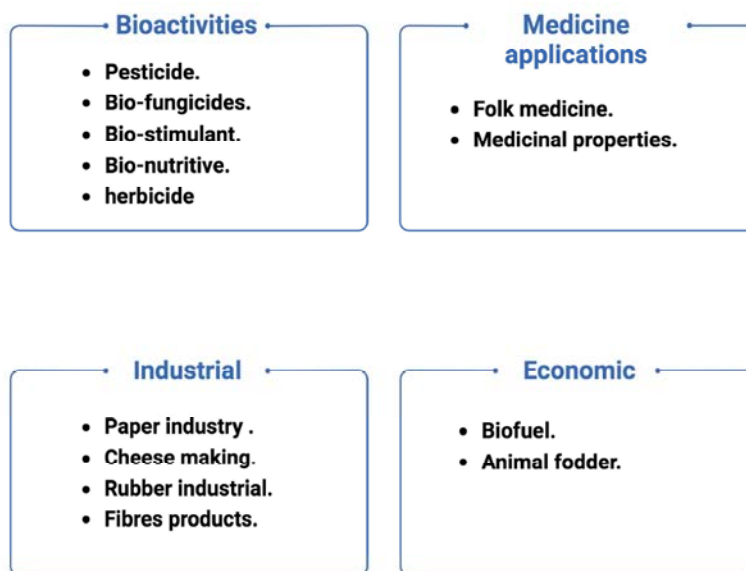


Fig. 3: The application of *C. procera*

cysteine peptidases (CpCPs) could coagulate bovine milk and generate cheeses with high yields. Likewise, research showed that the nutrient compositions are higher in cheese compared to other milk coagulants, as *C. procera* had higher values for lipids, protein, sugar, zinc, and copper [73]. More recently, CpCP3 (a cysteine peptidase purified from *C. procera* latex) exhibited high milk-clotting activity, and sensory evaluation revealed that cheeses produced with this enzyme had high acceptance [75]. In a comparative study between soluble CpCPs and immobilized CpCPs on glyoxyl-agarose (glyoxyl-CpCPs) were found to have a similar ratio of milk-clotting activity to proteolytic activity in comparison with soluble CpCPs; besides, even after six months of storage at 8°C, the proteolytic activity of glyoxyl-CpCPs remained close to 100 % [76].

Manufacture from *C. procera* Fibres: *C. procera* contains different types of fibers involved in manufacture. These fibers are found in different *C. procera* organs, and the inner bark of *C. procera* is used to produce the strong fibers known as madar in India, which are used to create carpets, harnesses, stitching thread, and fishing netting [77]. Its stem is a natural source of cellulosic bast fibers with commercially valuable properties, such as cellulose content, fiber strength, intermediate fiber elongation between cotton and linen, high tensile strength, and abrasion resistance. Due to its antimicrobial properties, bast fiber from *C. procera* can be substituted for cotton in surgical or structural applications [78]. The *C. procera*

fiber is of very high quality, as it contains 81.8% holocellulose and only 12% ash and 6.0% lignin. Therefore, it is an excellent alternative basic material for creating eco-friendly handmade paper [79, 80]. Moreover, the *C. procera* has short fibers inside the fruit and the seed floss; the advantages of this fiber, with its brittle, smooth and slippery surface, are that it can provide suitable composites to be used for stuffing purposes and produce yarn containing 100% fiber with a special procedure [72, 81].

The *C. procera* fibers were found to have the ability to adsorb the toxic organic dyes used in textile industry processes that are released as polluted wastewater, Thamer *et al.* [82] found that the carbon fibers and flakes derived from *C. procera* were efficient adsorbents for the removal of cationic crystal violet (CV) dye from aqueous solutions. Moreover, reports indicate that *C. procera* fiber can also be used as a bio-sorbent for the removal of oil spill-related contaminants [83, 84].

Construction of Nanoparticles: Green nanotechnology is an emerging discipline for the cost-effective and environmentally friendly production of metallic nanoparticles for a variety of industrial applications. *C. procera* has effectively enabled their production. *C. procera* leaf extract has been used as a reducing and stabilizing agent, and iron nanoparticle preparation has been optimized. Subsequently, the potential antifungal activity of iron nanoparticles was evaluated. The results showed that iron nanoparticles significantly reduced

the growth of *Alternaria alternata*. These findings demonstrate the high effectiveness, low cost, and environmental friendliness of the iron nanoparticles made from *C. procera* leaf extract. These excellent results point to the widespread application of these green nanoparticles as a potent substitute for chemical fungicide [85].

Anthelmintic present in ethanolic leaf extract of *C. procera* was used to synthesize its green-synthesis derivative utilizing silver nitrate (CP-AgNPs) to study its efficacy against the eggs besides miracidia of Fasciola species. The results showed that within 30 minutes of exposure to all tested concentrations, 100% mortality of miracidia was observed. This suggests the *C. procera* leaf extracts may be a potential source of antifasciola agents [86]. In a study done by Nagime *et al.* [87], the potential biological applications of *C. procera* flower extract, capped and stabilized silver nanoparticles (CP-AgNPs) synthesis were examined. They found that CP-AgNPs exhibited antibacterial and antifungal activity against pathogenic microorganisms. Furthermore, CP-AgNPs exhibited significant anti-diabetic and anti-inflammatory activity *in vitro*. In addition, a practical and efficient method for synthesizing AgNPs from *C. procera* flowers extract with enhanced biomimetic properties has been developed, with potential applications in water treatment, biosensors, biomedicine, and allied sciences.

CONCLUSION

The plant *Calotropis procera* is a valuable species for both medicine and the economy due to its diverse biological properties. This review aims to highlight important biological and ecological characteristics, applications in both conventional and cutting-edge domains. It is also an effort to identify the knowledge gaps and less-explored areas in further studies. Even if the morphological characteristics of the plant and its pharmacological applications and biochemical profile have gotten enough attention. Its overall biological and ecological characteristics have not received enough attention. Furthermore, little attention has been paid to the toxicity-bioactivity related to *C. procera*, which is crucial to substantiating its agricultural features. Assessing these fundamental aspects could enhance its economic use and open up new application opportunities.

At the same time, filling in these knowledge gaps can be beneficial to determine the plant's greater pharmacological and therapeutic qualities particularly every portion of the plant is abundantly supplied with a wide variety of phytochemical components, however,

additional laboratory and clinical studies are required. This will help in the creation of significant medications derived from *Calotropis procera's* active phytochemical ingredients.

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