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REVIEW ARTICLE

Important insights from the antimicrobial activity of *Calotropis procera*

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Abstract *Calotropis procera* (family Apocynaceae) is a valuable medicinal plant as it contains many valuable phytochemicals such as glycosides (mostly cardenolides), flavonoids, triterpenes, alkaloids, steroids, saponins, proteins and enzymes. Multiparous biological activities such as anti-inflammatory, antioxidant, anticancer, wound healing and wideranging antimicrobial activities of *C. procera* have been well investigated and reported. The main aim of this review was to present the encompassing information regarding antimicrobial activities of *C. procera* latex, different crude extracts and some isolated compounds which have been tested for antimicrobial property. Comprehensive data extracted from earlier as well as recently published original articles regarding antibacterial, antifungal, anti-protozoal and antiviral properties of *C. procera* were discussed and summarised in tabular forms. The compiled data comprised of plant parts, geographical origin, type of tested extracts/fractions, test model, used doses, tested microorganisms, obtained results and relevant references. In addition, the isolated antimicrobial pure compounds of *C. procera* are also dis-

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cussed in a separate section. The analysis and information presented in this review identified the existing critical knowledge gaps in the research and also explored the future perspectives and further research opportunities of *C. procera*.

Contents

1. Introduction

Plants, which have been the unique source of remedies for thousands of years, have been used in management of human's as well as animals' diseases (El-seedi et al., 2019). Currently, medicinal plants (MPs) are still the major source of primary health care in developing countries (Mulat et al., 2020). As per the World Health Organisation (WHO), around 80% of world populace especially in developing countries rely on traditional medicines, particularly on MPs for their routine health problems (Fatima et al., 2018; Jamshidi-Kia et al., 2018; Amini et al., 2019). However, only around 50% of western drugs contain plants bioactive compounds or their analogues as their active ingredients (Gupta & Pandey, 2020).

Microbial infections have been the main cause of mortality, and resistant microorganisms are increasingly threatening the public health worldwide (Vidyasagar, 2016; Khameneh et al., 2019; Biharee et al., 2020). Currently, the annual number of deaths reaches 700,000 due to resistant pathogens out of which around 230,000 deaths occur only due to Multidrug-resistant (MDR) tuberculosis. The drug-resistant diseases are expected to cause 10 million deaths per year by 2050 (Biharee et al., 2020). Similarly, the incidence of fungal infections has increased dramatically since the past few decades that can be attributed to the abundant spread of fungal spores in the soil and in the air. Exposure to heavy fungal spores can cause several infections (e.g., sinusitis, lung, and skin infections) particularly in immunocompromised individuals (Vidyasagar, 2016).

Development of new drugs and newer strategies are strongly needed to combat resistance to antibiotics (Khameneh et al., 2019; Mulat et al., 2020). The WHO emphasises on discovery of new antimicrobial drugs against the resistant pathogens (WHO, 2019). Phytochemicals have shown different degrees of activity against microbial pathogens, and they are believed to produce no or lesser side effects when compared to synthetic antimicrobials (Konaté et al., 2012; Vidyasagar, 2016; Pathania et al., 2020). Some phytochemicals can reverse or modify the antimicrobial resistance (Chusri et al., 2009), or may produce synergistic effects with conventional antibiotics (Lee et al., 2010). Indeed, phytochemicals may act as antimicrobial agents through different mechanisms (Biharee et al., 2020). That is to say, co-administration of antibiotics with the non-antibiotic compounds that act as resistance breakers, could be one of the useful strategy to enhance or restore antibiotics' activity (Chusri et al., 2009; Khameneh et al., 2019).

Calotropis procera (*C. procera*) is a popular medicinal plant from the family Apocynaceae. It is a xerophytic perennial shrub (or small tree) with stems of 2 to 6 m tall and tap roots 3 to 4 m deep in the soil (Hassan et al., 2015). A thick milky

Table 1 Overall information about the medicinal plant *Calotropis procera*.

Common/vernacular name	Country/ Language	Parts used/preparation	Disease	Reference
Ushar	Sudan: Arabic	Lt: paste (topical). Lf: powder, decoction, infusion (as mouthwash) or mixed with oil (topical). Fresh Rt: crushed or powdered (topical).	Skin or cutaneous illness: haemorrhoids, skin injuries, and scorpion bits. Rheumatoid pains, mouth infections, jaundice, and asthma.	(Mahmoud et al., 1979b; El-kamali, 2009; Salem & Algalib, 2011; Suleiman, 2015; Karar & Kuhnert, 2017)
Ushur, ushar	Yemen: Arabic	Lf: pasted	Skin and dermal illness: skin infections, boils and scabies.	(Fleurentin & Pelt, 1982; Ali et al., 2001; Al-Fatimi, 2019)
Akra, Akundia, Akonda or Akond, Akada, Akauwa, Rui, mandara, alaka, ravi, vellerukku,	India: Hindi, Bangali, Marathi, Tamil, Sanskrit	Lf, Rt, RtBk, StBk, Bd, Lt, FL: powdered, pasted, decoction, ashed (topical and oral). Rt: powdered + sugar (orally) RtBk: powdered + honey (orally)	Skin and dermal illness: elephantiasis, wounds, cuts, thorn injuries, inflamed swellings, ulcers, boils, ringworm, leukoderma, and leprosy. GIT illness: helminthiasis, diarrhea, dysentery and cholera. Malaria, fever, pain, jaundice, leucorrhea.	(Basu & Nag Chaudhuri, 1991; Samvatsar & Diwanji, 2000; Sharma & Sharma, 2000; Panda et al., 2011; Sharma et al., 2011; Dubey et al., 2012; Samy & Chow, 2012; Bhatia et al., 2014; Gairola et al., 2014; Panda, 2014; Payal & Sharma, 2014; Sharma et al., 2014)
Ushaar, oshar, usher, Kisher,	Saudi Arabi: Arabic	ArPt, FL, Lf, Lt, RtBk, St, Rt: powdered, decoction, liniment, paste (oral and external.	Skin and dermal illness: infections, leprosy, wounds, psoriasis, boils, leishmaniosis, scorpion stings and hair loss. GIT diseases: dysentery, constipation, worms and toothache. Respiratory diseases: bronchial asthma and cough. Malaria, fever, headaches, joint pain, rheumatism, and muscular spasms.	(Mossa et al., 1991; Al-Qarawi et al., 2001; Al-Mezaine et al., 2008; Gherbawy & Gashgari, 2013; Tounekti et al., 2019)
Bunagadhee, Ttobia	Ethiopia	Rt, Lf, Lt: alone or mixed with other plants (topically).	Skin diseases: tropical ulcers, wounds, infections, boils (furuncle).	(Desta, 1993; Wondimu et al., 2007; Meragiaw et al., 2016)
Al-Ashkar	United Arab Emirates: Arabic	Not specified	External usages to relief inflammations	(Tanira et al., 1994)
Baniwani, kipanpango	Gambia: Jola language	Lf	Toothache, sore hands	(Madge, 1998)
Aldebaj	Iraq: Arabic	Bk: decoction (orally)	Tonic, sudorific, antispasmodic, expectorant, and emetic (large doses)	(Al-douri, 2000)
Flor de seda, ciúme, ciumeira,	Brazil: Portuguese	Lt: as paste (topically)	Skin/dermal diseases: infections	(Alencar et al., 2004; Lázaro et al., 2012)
Fogofoko, Anranpobo, Pumpum, Pompo pokolo	Mali	Lf: crushed (topical), decoction (orally and bath)	Headache, muscular pains, pain because of sickle cell disease, malaria	(Inngjerdingen et al., 2004; Diarra et al., 2015; Danton et al., 2019)
Tumfafiya, Bomubomu and Kayou	Nigeria: Hausa and Yoruba languages	Wp, Lf, St: decoction, ashed, burned or smoked. Lt: fresh paste or with honey	Skin/dermal diseases: eczema, ringworms, fungal infections e.g., *Tinea capitis*. GIT diseases: indigestion, diarrhoea and toothache, Respiratory illness: cough. Fever, rheumatism, rabies (Lt + honey)	(Adamu et al., 2005; Iwalewa et al., 2005; Kuta, 2008; James et al., 2013; Aliyu et al., 2015; Abubakar et al., 2020)
Putrepuugu	Burkina Faso	Different parts. Rt: boiled with white stones and cowry shell (decoction as mouthwash)	Neuropsychiatric disorders, liver diseases, malaria, tumour and tooth pain.	(Tapsoba & Deschamps, 2006; Kinda et al., 2020)
Spalmai or Spalmey,	Pakistan:	Lf: crushed alone or	Skin/dermal diseases: wounds,	(Husain et al., 2008; Abbasi et al.,

(*continued on next page*)

Table 1 (*continued*)

Common/vernacular name	Country/ Language	Parts used/preparation	Disease	Reference
Spalmaka, Aak	Pushto, Urdu	mixed with oil (topically). Lt: mixed with other plants or mustard oil or flour (topically). RtBk, FL, FR, Lf, St, Rt: alone or mixed with other plants. Decoction, infusion, and powdered (Oral and topically). Rt: Smoked (inhalation) and ashed.	scabies, eczema, lice, ringworms, snake and scorpion bites, carbuncle. Respiratory diseases: cold cough, asthma, pneumonia. GIT diseases: mouth and dental infections, toothache, cholera, diarrhoea, abdominal pain. UT diseases: kidney stones and chronic renal problems. Jaundice, malaria, fever, earache.	2010; Ullah et al., 2010; Azhar et al., 2014; Ahmed et al., 2015; Butt et al., 2015; Rehman et al., 2015; Aziz et al., 2017; Shah & Rahim, 2017; Fatima et al., 2018; Hussain et al., 2018; Bahadur et al., 2020; Hassan et al., 2020; Manduzai et al., 2021)
Akondo gach	Bangladesh	Lf: warmed and (topically applied to the painful part of body)	Body pain	(Rahmatullah et al., 2010b)
NR	Thailand	Lf: grounded and paste (topically)	GIT diseases: aphthous ulcers and lesion	(Neamsuvan et al., 2012)
Punpune	Ghana	Rt: poultice (topically)	Skin/dermal diseases: boils	(Wodah & Asase, 2012)
Göbi	Guinea: pular or fula	Lf: decoction (orally)	Malaria	(Traore et al., 2013)
Kebou	Kenya	Lf: ashed (orally) FL: decoction (orally)	Malaria, and as emetic.	(Kipkore et al., 2014; Muthaura et al., 2015)
Kharak	Iran: Persian	Lf, Lt, Rt: decoction, dressing (topically)	Skin/dermal diseases: inflammations, snake, scorpion and insect bites. Gastric discomforts, and migraine.	(Sadat-Hosseini et al., 2017)
Tourjah	Mauritania: local Arabic	Lf: powdered + honey and olive oil (orally).	Respiratory diseases: whooping cough	(Yebouk et al., 2020)
Tourja	Morocco: Darija	St: decoction (topically)	Skin/dermal diseases: wounds	(Idm'hand et al., 2020)

Abbreviations: Bd; bud, Bk; bark, Lf; leaf, Lt; latex, FL; flowers, FR; fruits, NR; not reported, Rt; roots, RtBk; root bark, St; stem, StBk; stem bark, Wp; whole plant.

sap or latex exudes out from the plant if its parts are cut or broken (James et al., 2013; Waikar & Srivastava, 2015). *C. procera* grows on a variety of soils and it can tolerate different level of soil salinity, draught stress, intense light of arid and harsh environments. Hence, it is distributed in various tropical and subtropical countries (Hassan et al., 2015).

C. procera has been known as medicinal plant for a long time (Al-Sulaibi et al., 2020), and it has been used in treatment of a diverse array of maladies and particularly infectious diseases (Oraibi & Hamad, 2018; Pathania et al., 2020) (Table 1). Moreover, *C. procera* has been worshiped by ancient Indians and grown near temples (Sharma & Sharma, 1999), used as milk-clothing agent in preparation of the African local cheese called *wagashi* (Belvedere et al., 2010).

C. procera latex (CPL) and its different parts contain various metabolites such as glycosides and cardenolides (Mohamed et al., 2015; Sweidan & Zarga, 2015), flavonoids (Mendki et al., 2005), triterpenoids (Khan et al., 1988; Gupta et al., 2002;), steroids (Khan & Malik, 1989), saponins (Gupta et al., 2002, 2003), lignans (Abdel-Mageed et al., 2016; Al-Taweel et al., 2017), proteins and different enzymes (Lima-Filho et al., 2010; Kumar et al., 2015; Bezerra et al., 2017; Freitas et al., 2020), hydrocarbons (Erdman & Erdman, 1981), saturated and unsaturated fatty acids (Khanzada et al., 2008; de Sousa et al., 2018).

C. procera showed a diverse array of biological activities such as antimicrobial (Yesmin et al., 2008; Velmurugan et al., 2012; Tiwari et al., 2016), antidiarrhoeal (Kumar et al., 2001), wound healing (Aderounmua et al., 2013; De Figueiredo et al., 2014), anti-inflammatory (Alencar et al., 2004; Kumar et al., 2011; Ramos et al., 2020), anticancer or cytotoxic (Samy et al., 2012; Mohamed et al., 2015; Chan et al., 2017), *in vivo* immunomodulatory (Nascimento et al., 2016), analgesic (Basu & Nag Chaudhuri, 1991; Pathak & Argal, 2007), anthelmintic (Shivkar & Kumar, 2003; Iqbal et al., 2005), antioxidant (Yesmin et al., 2008), and *in vivo* anti-hyperglycemic (Roy et al., 2005; Rahmatullah et al., 2010a).

Although ethnobotanical uses, phytochemistry and different biological potentials of *C. procera* have been partially reviewed by other authors (Silva et al., 2010; Chan et al., 2016, 2017; Mali et al., 2019; Shamim et al., 2019; Ali-Seyed & Ayesha, 2020; Pathania et al., 2020), there remains the lack of comprehensive review of *C. procera* antimicrobial properties. Therefore, in this review, efforts were made to present a comprehensive and state of the art data regarding antibacte-

rial, antifungal, anti-protozoal and antiviral properties of CPL, its different extracts, fractions and isolated compounds and fungal endophytes which were evaluated for antimicrobial activities. In addition to the compilation of traditional uses of *C. procera* in different countries (Table 1), we have also compiled elaborated data regarding antibacterial, antifungal, anti-protozoal, and antiviral activities of CPL and its different crude extracts in tabular forms. The tabulated data including the plant parts, geographical origins, types of extracts, test model, dosage, tested microorganisms, and results are presented in Tables 2–5. In addition, *C. procera* isolated compounds which have been tested for antimicrobial potential, were also highlighted separately, while future perspective and research opportunities of *C. procera* were also discussed in the current review paper.

To collect the required information for this manuscript, published articles were searched through different websites e.g., Academia, Google Scholar, PubMed, Research Gate, Science Direct, Web of Science, websites of different open-access journals, etc. using appropriate keywords such as traditional uses of *C. procera*, antimicrobial/bacterial effects of *C. procera*, antiviral effects of *C. procera*, fungicidal or antifungal effects of *C. procera*, wound healing effects of *C. procera*, antiprotozoal properties of *C. procera*, antimicrobial phytochemicals/compounds of *C. procera*, fungal endophytes of *C. procera*, etc. Although a huge number of articles were collected and read, only those focusing on antimicrobial activity of *C. procera* were selected, reviewed and used/cited for compilation of necessary data for present review paper.

Based on our literature survey, a huge number of publications reported the antimicrobial activities of CPL and *C. procera* crude extracts, but limited works regarding microbiological properties of *C. procera* isolated compounds were published. Moreover, data relevant to the antimicrobial mechanism of actions (MOA) of *C. procera* was very scarce. Therefore, further in-depth investigations are encouraged to explore *C. procera* antimicrobial compounds and their MOA.

1.1. Geographical distribution of C. procera

C. procera is native to North and Tropical Africa, Western and South Asia and Indochina up to the Arabian Peninsula, and it is widely distributed in Australia, American countries and West indies (Chan et al., 2017; Mutwakil et al., 2017). Being able to grow in both dry and wet environments, the plant develops a wide range of morphological traits, and is found as different morphotypes. The deep and stout taproot system of *C. procera* enable the plant to grow and survive in dry dessert areas (Pompelli et al., 2019). *C. procera* grows in different countries such as Afghanistan, Algeria, Australia, Bangladesh, Bolivia, Brazil, Democratic Republic of Congo, Cameroon, Chad, Chile, China, Colombia, Cuba, Ecuador, Egypt, Eritrea, Ethiopia, Ghana, Guatemala, Guinea-Bissau, Haiti, Jamaica, India, Israel, Jordan, Lebanon, Libyan, Malaysia, Mali, Mauritania, Mexico, Mozambique, Myanmar, Morocco, Nepal, Netherlands Antilles, Nicaragua, Nigeria, Pakistan, Panama, Paraguay, Peru, Puerto Rico, Saudi Arabia, Senegal, Somalia, Sudan, Tanzania, Uganda, United Arab Emirates, Uruguay, Venezuela, Yemen and Zimbabwe (Carruthers et al., 1984; Basu & Nag Chaudhuri, 1991; Mossa et al., 1991; Lev-yadun, 1999; Alencar et al., 2004; Lottermoser, 2011; Breckle et al., 2013; Traore et al., 2013; Azhar et al., 2014; Diarra et al., 2015; Suleiman, 2015; Chandrawat & Sharma, 2016; Meragiaw et al., 2016; Fatima et al., 2018; Al-Fatimi, 2019; Gracia et al., 2019; Shamim et al., 2019; Idm'hand et al., 2020; Yebouk et al., 2020).

1.2. Traditional medicinal importance of C. procera

C. procera, as an ancient medicinal plant, has been known to Greeco-Arab medicine since long time ago, and ancient Egyptians have used it in Neolithic period in Egypt (Hassan et al., 2015). It is a famous medicinal plant of Ayurveda, Arabic, Siddha, Unani and Sudanese traditional medicines (Sharma & Sharma, 1999; Oraibi & Hamad, 2018; Pathania et al., 2020), and is called by several common and vernacular names such as Giant Indian milked weed, Madar and Sodom apple (English), Ak or Arka (Hindi, Sanskrit), Remiga (Malay), Rubik (Indonesian), Ipekag (Turkish), Oshar or Ushar (Arabic), Kharak (Persian), and Spalmai (Pushto) (Breckle et al., 2013; Parihar & Balekar, 2016; Sadat-Hosseini et al., 2017; Tounekti et al., 2019; Bahadur et al., 2020; Manduzai et al., 2021). In different countries, the CPL and almost all parts of *C. procera* have been used traditionally as multifarious remedies for several medicinal purposes (Table 1).

As shown in Table 1., different parts of *C. procera* and CPL have been traditionally used by people in different countries (21 countries in total) for treatment of various health problems including tumours, jaundice, body pains, fever, various infections, and so forth (Mascolo et al., 1988; Basu & Nag Chaudhuri, 1991; Sharma & Sharma, 1999; Kumar et al., 2005; Murti et al., 2010; James et al., 2013; Tounekti et al., 2019). It is worth noting that *C. procera* has been used more frequently in treating various infectious diseases that could be broadly classified into five categories of (1). skin and dermal infections (e.g., leprosy, wounds and skin infections, boils, carbuncles, scabies, leishmaniosis, mouth and dental infections), (2) gastro-intestinal tract (GIT) infections (e.g., dysentery, diarrhoea, cholera, gastritis, colitis and worms), (3) respiratory infections (bronchitis, bronchial asthma, cough and pneumonia) and (4) gyneco-urinary infections (chronic renal problems and leucorrhea), and (5) systemic infection (e.g., malaria and elephantiasis).

C. procera has been used both as external (topical) and internal (oral) preparations. However, its external or topical uses were more dominant considering its higher usability in the management of dermal infections, wounds, cuts, wasp stings, psoriasis, eczema, scorpion and snake bites, body pains, and so on (Table 1).

1.3. Toxicity of C. procera

Apart from its proven traditional use in various countries (Table 1), *C. procera* is also enlisted as weed (Gracia et al., 2019), and as a toxic plant (Tossou et al., 2018; Al-Zuhairi et al., 2020). Ingestion of CPL and fresh leaves of *C. procera* by ruminants has caused toxic effects to the animals (Mahmoud et al., 1979a, 1979b). Once the plant was used as abortifacient as well. Toxicity of the plant is principally due to presence of toxic compounds such as toxic cardenolides in its latex and all other parts of the plant.

Table 2 Antibacterial activity of *C. procera* latex and different extracts.

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Flowers	India	80% EtOH ext.	*In vitro*: DDM	500 µg/disc	G–ve: *E. coli* 7075, *E. coli* Bb, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* H G + ve: *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Bacillus anthracis*, *Bacillus subtilis*	Active against all tested bacteria (except *P. aeruginosa*) showing ZOI of 16.5–26.1 mm and MICs of 2.7–4.0 mg/mL. *S. typhi* H and *S. aureus* were the most susceptible bacteria.	(Mascolo et al., 1988)
CPL	Ethiopia	Aq. ext. and PetE, CHL, MeOH Fct. of 80% EtOH ext.	*In vitro*: WDM	200 µg/well	G–ve: *Salmonella gallinarum* (ATCC 9184), *E. coli* (ATCC 9637), *P. aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (isolate) G + ve: *S. aureus* (ATCC 13709)	Active on all tested bacteria. The crude aqueous extract was more potent than the organic fractions of EtOH extract.	(Desta, 1993)
Leaves, stems, roots, flower, fruit, root-barks, CPL	India	EtOH ext.	*In vitro*	NF	G–ve: *Enterobacter cloacae* (*E. cloacae*), *E. coli,* G + ve: *S. aureus, Streptococcus faecalis* (*S. faecalis*).	Among tested samples, root-barks showed maximum effects on *E. cloacae*. CPL did not show activity	(Jain et al., 1996)
Leaves	Yemen	EtAc fraction of 80% EtOH ext.	*In vitro*: DDM	4 mg/disc	G–ve: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) G + ve: *S. aureus* (ATCC 29213), *S. aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212)	EtAc fraction produced ZOI of 3–5 mm (excluding disc diameter) only against G + ve strains. The G–ve were not susceptible.	(Ali et al., 2001)
Leaves, flowers	Morocco	EtOH, EtAc, and n-BtOH ext.	*In vitro:* DDM for ZOI, TDM for MIC	500 µg/disc	G–ve: *E. coli, K. pneumoniae, P. mirabilis, S. typhi, Shigella flexneri, Enterobacter aerogenes* G + ve: *Corynebacterium pyogenes, S. aureus*	Flower ext. was more potent. n-BtOH ext. of flower was active on all tested bacteria (ZOI; 11.3 ± 0.5–18.3 ± 0.5 mm), except *E. aerogenes*. The *E. coli, S. typhi*, *S. aureus* showing ZOI of 16–18 mm were most susceptible strains to flower BtOH ext., with MIC of 4 and 5 mg/mL against *E. coli* and *K. pneumoniae*, respectively. EtOH ext. of flower was active against all tested bacteria (ZOI; 7.9 ± 1.2–12.3 ± 0.3 mm) except *S. flexneri*, while *K. pneumoniae, E. coli* and *S. typhi* were most sensitive (ZOI; 10.5–12.3) to EtOH flower ext. Leaf EtAc ext. produced ZOI of 11.3–13.5 mm against *E. coli*, *P. mirabilis* and *K. pneumoniae*. Remaining ext. had lower activity only against some of tested strains.	(Larhsini et al., 2001)
Whole plant	Nigeria	Aq. ext.	*In vitro:* WDM	200 mg/mL	G–ve: *P. mirabilis, P. aeruginosa, E. coli* G + ve: *S. aureus*	Mild activity with ZOI < 15 mm.	(Adamu et al., 2005)

Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Roots	Nigeria	Aq. ext.	*In vitro:* DDM	Discs soaked in 60, 50 and 40% ext.	G–ve: *E. coli, Neisseria gonorrhoeae* G + ve: *S. aureus*	Mild activity with ZOI < 9 mm. *N. gonorrhoeae* was more susceptible (ZOI: up to 9 mm). *E. coli* was resistant.	(Filgona et al., 2005)
Leaves	India	EtOH ext.: partially purified Flv mixture	*In vitro:* WDM	70 µg/mL	G–ve: *P. aeruginosa*, *Pseudomonas putida*. G + ve: *Bacillus megaterium, B. subtilis, Cellulomonas uda*.	Flavonoid's fraction showed ZOI of 10.66 ± 1.33–15.66 ± 0.33 mm against tested bacteria except *P. aeruginosa* that was less susceptible. *Cellulomonas uda* was most susceptible.	(Mendki et al., 2005)
Leaves, stems	Nigeria	50% EtOH ext. and its CHL, EtAc and BtOH Fct.	*In vitro*: WDM	Crude ext. at 20 and 40 mg/mL. Fractions at 5 mg/mL	G–ve: *E. coli, K. pneumonia, S. typhi* G + ve: *B. subtilis, S. aureus*	Leaf ext. at 20 mg/mL showed ZOI of 9–11 mm, and *S. aureus* was more susceptible. Stem ext. at 20 mg/mL showed ZOI of 10–14 mm, *S. aureus* was more susceptible. Leaf fractions showed ZOIs; 10–16 mm, *S. aureus* was most sensitive. Stem fractions showed ZOI; 10–18 mm, *S. aureus* and *B. subtilis* were more susceptible. *E. coli* was less susceptible in all cases.	(Oladimeji et al., 2006)
Leaves, CPL	Nigeria	60% EtOH ext.	*In vitro*: WDM	50 µL/well (Eq. 5 mg RM/well)	Clinical isolates: G–ve: *E. coli, P. aeruginosa* G + ve: *Staphylococcus albus, S. aureus, Streptococcus pyogenes, Streptococcus pneumoniae*	EtOH ext. of CPL was more potent (ZOI: 7.0–14.1 mm and MIC: 2.5–7.5 mg/mL) than the leaf extract (ZOI: 3.0–8.5 mm and MIC: 5–12.5 mg/mL). *E. coli* was the most susceptible bacterium.	(Kareem et al., 2008)
Twigs, CPL	India	Different fractions of Aq. decoction	*In vitro:* DDM	4 mg/disc	8 Opportunistic bacteria: G–ve: *P. aeruginosa, K. pneumoniae, Serratia marcescens* (*S. marcescens*), *E. aerogenes*, *E. coli*. G + ve: *S. aureus, S. epidermidis, Bacillus cereus* 2 pathogenic strains: G–ve: *Salmonella paratyphi* A and *S. typhi*. 2 non-pathogenic: G + ve: *B. subtilis*, *M. luteus*	Different range of activity was observed. All ext. and fractions were active showing ZOI of 5–27 mm. EtAc Fct. of twigs being the most potent against all tested bacteria showed ZOI of 13–27 mm. EtAc Fct. of CPL was the 2nd potent sample with ZOI of 13–20 mm against all test strains. *S. aureus* was most susceptible to EtAc Fct. Water ext. was least active sample (ZOI; 5–10 mm).	(Parabia et al., 2008)
Leaves	Bangladesh	90% MeOH and Aq. ext.	*In vitro*: WDM	500 µg/well	G–ve: *Shigella dysenteriae, S. flexneri, Shigella sonnei, V. cholerae* G + ve: *S. aureus, S. epidermidis, Staphylococcus saprophyticus, S. pyogenes*	Aq. ext. was more potent with ZOI of 10–22 and 7–10 mm against G + ve and G–ve, respectively. MeOH ext. produced ZOI of 6–9 mm only against few bacteria.	(Yesmin et al., 2008)
Seeds	India	CHL and MeOH ext.	*In vitro*: DDM	Disc dipped in 1 mg/mL solutions	G–ve: *Aeromonas hydrophila* (*A. hydrophila*) ATCC 79, *E. coli* (MTCC 118), *Morganella morganii* (ATCC 102), *P. vulgaris* (MTCC 201) G + ve: *S. aureus* (MTCC 737), *B. subtilis*, *Mycobacterium smegmatis* (MTCC 106)	CHL and MeOH extracts produced ZOI of 10.21–15.35 and 09.15–12.35 mm, respectively, against the tested bacteria, and *S. aureus* was most susceptible.	(Bhaskar & Ajay, 2009)

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Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Leaves, CPL	Nigeria	95% EtOH ext.	*In vitro*: DDM, MIC	Disc soaked in 1–10 mg/mL	G–ve: *E. coli, Pseudomonas* sp., *Salmonella* sp. G + ve: *S. aureus*	Dose dependent activity was observed. Leaf ext. showed ZOI of 7–10 mm and MIC of 2 mg/mL for *S. aureus* and ZOI: 9–15 mm and MIC of 1 mg/mL for *E. coli*. CPL ext. showed ZOI of 8–10 mm and MIC of 2 mg/mL against *E. coli*. *Salmonella* sp. and *Pseudomonas* sp., were not sensitive.	(Kawo et al., 2009)
Leaves	India	MeOH, CHL, EtAc, and Aq. ext.	*In vitro*: WDM	20–50 µg/well	G–ve: *S. typhi* (MTCC 734), *S. paratyphi* A (MTCC 3220), *V. cholerae* (MTCC 3904), *K. pneumoniae* (MTCC 109)	Dose dependent effects was produced. EtAc ext. was most potent against all bacteria (ZOIs of 9 ± 1.5–22 ± 1.3 mm) except *K. pneumoniae*, while *Salmonella* sp. was most susceptible to EtAc ext. MeOH ext. was only active on *K. Pneumoniae* (ZOI; 17 ± 1.6 mm). *V. cholerae* showing ZOI: 13 ± 1.4 mm was the most susceptible bacterium to CHL ext.	(Mohanraj et al., 2010)
Leaves	Nigeria	ACT ext.	*In vitro*: MDM	10 mg/mL and 2fd	G + ve: *M. mycoides*	Showed strong effect with MIC of 80 µg/mL and MMC of 160 µg/mL.	(Muraina et al., 2010)
Leaves, flowers	Pakistan	80% EtOH, 80% MeOH, and 80% ACT ext.	*In vitro*: DDM	1500 µg/disc	G–ve: *E. coli* DH5α G + ve: *Bacillus pumilis* JF313263	Leaves' ext. showed ZOI of 14.9 and 19.2 mm, and MIC of 21 and 28 mg/mL against *E. coli* and *B. pumilis*, respectively. Flower ext. showed ZOI of 12.3 and 17.6 mm, respectively. EtOH ext. was more potent, while the MeOH and ACT extracts produced milder antibacterial effects.	(Ahmad et al., 2011)
Flower, buds, leaves, stems	India	EtOH ext.	*In vitro*: DDM	5 µg/disc	G–ve: *E. coli, S. typhi, P. aeruginosa, S. marcescens* G + ve: *B. cereus, B. subtilis, S. aureus, M. luteus*	Leaf ext. produced ZOIs of 7–15 and 5–10 mm against G + ve and G–ve, respectively. Bud ext. produced ZOI of 8 mm only against *S. aureus*. Flower and stem extracts were not active.	(Doshi et al., 2011)
Flower	Pakistan	70% MeOH ext.	*In vitro*: DDM, ADM	10 mg/disc	G–ve: *Pasteurella multocida, E. coli* (ATCC 29922). G + ve: *B. cereus, Corynebacterium bovis, S. aureus* (ATCC 29923)	The ext. showed ZIO of 12 ± 0.24–18 ± 0.18 mm and MIC of 6.25–25 mg/mL against tested bacteria. *C. bovis* was most susceptible while *S. aureus* was not susceptible.	(Hussain et al., 2011)
Fruits, barks	Nigeria	Aq., MeOH, and 95% EtOH ext.	*In vitro*: WDM	30 –120 mg/mL	G–ve: *P. aeruginosa, S. typhi, E. coli* G + ve: *S. pyogenes*	Aq. ext. 30 mg/mL was active on tested bacteria. MeOH and EtOH ext. showed weak effects. *S. pyogenes* and *P. aeruginosa* were most susceptible strains.	(Mainasara et al., 2011)
Leaves, roots	Nigeria	Aq., MeOH, and 95% EtOH ext.	*In vitro*: WDM	30–120 mg/mL	G–ve: *P. aeruginosa, S. typhi, E. coli* G + ve: *S. aureus* and *S. pyogenes*	Leaf Aq. ext. showed broader effects. *S. pyogenes* and *E. coli* were most susceptible to leaf and root extract, respectively.	(Mainasara et al., 2012)

Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Leaves	India	Aq. and 80% EtOH ext.	*In vitro*: WDM	300 µg/well	G–ve: *Proteus* sp., *Citrobacter freundii*, *Chromobacterium violaceum*, *S. typhi*, *E. coli*, *Acinetobacter* sp., *Klebsiella* sp., *P. aeruginosa, V. cholerae* G + ve: MRSA	EtOH ext. showed ZOI of 10–18 mm against *S. aureus* and the first five listed G–ve bacteria. The remaining four G–ve were not susceptible. Aq. ext. was only active on *C. violaceum* (ZOI 11 mm).	(Dubey et al., 2012)
Leaves	India	Hex, EtAc, and MeOH ext.	*In vitro*: WDM	10–100 µg/well	G–ve: *P. aeruginosa, S. typhi, Vibrio harveyi, Photobacterium* sp., and *Aeromonas hydrophila*	EtAc ext. showed ZOI: 16.3 ± 1.24–24.8 ± 3.29 mm and MIC: 60–120 µg/mL. *V. harveyi* and *A. hydrophila* were the most susceptible strains. Hex. & MeOH ext. had mild and moderate effects, respectively.	(Velmurugan et al., 2012)
Stem-barks	India	EtAc, DCM, MeOH and Aq. ext.	*In vitro*: DDM	100 µg/mL	G–ve: *E. coli*, *E. aerogenes*, *P. mirabilis*, *P. vulgaris* G + ve: *P. aeruginosa, S. aureus*	EtAc, MeOH and Aq. extracts produced higher ZOI. The Aq. ext. was the most potent and showed ZOI of 9–28 mm. *S. aureus* was more susceptible. The calo-protein purified from Aq. extract showed strong effect (ZOI up to 30 mm) against *S. aureus*.	(Samy & Chow, 2012)
Leaves	India	EtOH, MeOH, and Aq. ext.	*In vitro*: WDM	1.25–5 µg/well	G–ve: *E. coli* (MTCC-40), *P. aeruginosa* (MTCC- 424). G + ve: *S. epidermidis* (MTCC-10623), and *B. subtilis* (MTCC 736).	All extracts showed strong activity at the highest dose. EtOH ext. showed ZOI of 11 ± 0.1–20 ± 1.0 mm, *P. aeruginosa* being most susceptible. MeOH ext. showed ZOI of 11 ± 0.5–14 ± 0.5 mm, *E. coli* being most sensitive. Aq. ext. showed ZOI of 13 ± 0.5–14 ± 0.5 mm, almost similar against all tested bacteria.	(Joshi & Kaur, 2013)
Leaves	Saudi Arabia	MeOH ext., Flv. Fct.	*In vitro*: WDM for ZOI and MIC	40 µg/well	G–ve: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (ATCC 13883), *Salmonella enteritidis* (ATCC 13076). G + ve: *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *B. subtilis* (ATCC 6633), *M. luteus* (ATCC 4698).	Crude ext. showed ZOI of 9.5–22.5 mm, and MIC of 0.16–1.28 mg/mL. Flv. Fraction showed ZOI: 15.5–28.5 mm, and MIC of 0.04–0.32 mg/mL. *B. subtilis* and *S. aureus* were the more sensitive strains.	(Nenaah, 2013a)
Leaves	India	Aq. and MeOH ext.	*In vitro*: WDM	800 µg/well	G–ve: *P. aeruginosa* (MTCC 1034), *Pseudomonas fluorescence* (MTCC 1748). G + ve: *S. epidermidis* (MTCC 3615)	Aq. ext. showed ZOI of 10–12 mm. MeOH ext. produced only ZOI of 11 mm against *P. fluorescence*.	(Panda, 2014)
Leaves, CPL	Egypt	CHL, EtOH, MeOH, 70% EtOH, and Aq. ext.	*In vitro*: DDM	Disc soaked with 20–50 mg/mL	G–ve: *S. typhi* (ATCC 19430), *E. coli* (ATCC 25922), *S. flexneri* (ATCC 12022), *E. faecalis* (ATCC 29212), *Neisseria lactamica* (ATCC 23970) G + ve: *S. aureus* CONS (ATCC 29213), MRSA (ATCC 43300).	EtOH and Aq. ext. were more potent. Aq. and EtOH ext. of leaves showed ZOI of 7–13 and 11–27 mm against tested bacteria, except *S. flexneri* and *E. faecalis*. Aq. and EtOH ext. of CPL produced ZOI of 7– 12.5 and 9–25 mm against tested bacteria, except against *S. flexneri* & *E. faecalis*. The most susceptible bacterium was *N. lactamica*	(Salem et al., 2014)

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Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Leaves, roots	Pakistan	90% EtOH and Aq. ext.	*In vitro*: DDM	0.5–10 mg/ disc	G–ve: *E. coli, P. aeruginosa,* G + ve: *S. aureus, S. pyogenes*	EtOH ext. was more potent. EtOH ext. of leaf at highest dose produced ZOI of 5.5–21 mm (*S. aureus* was more sensitive). EtOH ext. of root at highest dose produced ZOI of 5–18 mm (*S. pyogenes* was more sensitive). Aq. ext. of leaf at highest dose produced ZOI of 10–18 mm (*E. coli* was more sensitive). Aq. ext. of root at highest dose produced ZOI of 7–15 mm (*E. coli* was more sensitive).	(Mako et al., 2012)
Roots	India	EtOH ext.	*In vitro*: WDM, MDM	1 mg/well (for ZOI) 0.5–10 mg/ mL (for MIC)	G–ve: *E. coli* (NCIM 2931), *Pseudomonas* sp. (NCIM 5029), *Salmonella typhimurium* (NCIM 2501) G + ve: *B. subtilis* (NCIM 2545), *S. aureus*	It produced ZOI of 9–13 mm, and *Pseudomonas* sp. was more susceptible to the extract. *S. aureus* showed MIC of 1 mg/mL to the extract.	(Gajare et al., 2012)
Flowers	India	Hex, CHL, and MeOH ext.	*In vitro*: WDM	0.4–4 mg/ well	G–ve: *E. coli, K. pneumoniae,* G + ve: *B. subtilis, Lactobacillus acidophilus* (*L. acidophilus*), *S. aureus, S. epidermidis, Streptococcus gordonii, Streptococcus mutans* (*S. mutans*), *Streptococcus salivarius.*	MeOH ext. was more potent, and at dose of 2.4 mg/well produced ZOI of 10–11 mm. *K. pneumoniae* being most susceptible showed ZOI of 10 mm to MeOH ext. (0.2 mg/well). Hex extract at highest dose was active only on certain strains. CHL ext. at highest dose was active on all tested bacteria.	(Mastanaiah et al., 2012)
Flowers	India	70% EtOH ext.	*In vitro:* WDM	5–50 µg/ mL: (0.25–2.5 µg/well)	G–ve: *E. coli* (NCIM 2067), *P. aeruginosa* (NCIM 2037), *P. vulgaris* (NCIM 2027). G + ve: *B. subtilis* (NCIM 2063), *B. pumilis* (NCIM 2327), *M. luteus* (NCIM 2871), *S. aureus* (NCIM 2079).	At highest dose produced ZOI of 15.3 ± 0.5–18.3 ± 0.67 mm against tested bacteria. *S. aureus* and *P. vulgaris* were the most susceptible bacteria.	(Ranjit et al., 2012)
Flowers	Karachi, Pakistan	80% EtOH ext. and its Hex, BtOH, EtAc and Aq. Fractions	*In vitro*: WDM	100 µL/well	G–ve: *S. typhi*, *E. coli* G + ve: *M. luteus,* MRSA	Hex. Fct. was active against all tested bacteria with ZOI of 12–22 mm. EtAc Fct. showed ZOI of 15, 18 and 25 mm against *E. coli*, MRSA, *M. luteus*, respectively. Each of BtOH and Aq. Fct. produced ZOI of 30 mm only against *M. luteus*. *M. luteus* was most sensitive to all ext.	(Ali et al., 2014).
Aerial parts	India	MeOH ext.	*In vitro*: WDM	5 mg/well	G–ve: *Pseudomonas marginalis*, *Pseudomonas syringae* (MTCC 1604), *P. aeruginosa* (MTCC 1688), *Xanthomonas campestris* (MTCC 2286). G + ve: *L. acidophilus* (MTCC 447), *S. aureus*, *S. mutans* (MTCC 890), *S. salivarius* (MTCC 1938).	It showed ZOI of 9–21 and 10 –14 mm against tested G + ve and G–ve bacteria. *S. aureus* and *L. acidophilus* were the most and least susceptible strains, respectively.	(Vadlapudi et al., 2012)

Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
CLP	Egypt	Aq. solution of CPL serum	*In vitro:* WDM	300 µL	Clinical isolates: G–ve: *E. coli, P. aeruginosa, Serratia* sp.	It produced ZOI of 9.0–15.8 mm, and *E. coli* was the most susceptible bacterium.	(Mohamed et al., 2014)
Leaves, flowers, CPL	Saudi Arabia	Aq., 80% MeOH, and DiEE ext.	*In vitro*: DDM	20 µL/disc (for ZOI). 0.25–6 mg/mL for MIC	G–ve: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853). G + ve: *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *S. pneumoniae* (ATCC 49619), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 11778).	MeOH ext. showed strong and broad activity. MeOH ext. of flower and leaf showed ZOI of 10–18 and 11.5–18.5 mm and MIC of 1.5–3.0 and 0.25–2.5 mg/mL, respectively, while *B. cereus* and *E. coli* were the most susceptible strains. MeOH, Aq. and DiEE ext. of CPL showed ZOI of 11–23.5, 6.5–14 and 7– 12.5 mm and MIC of 0.25–3.0, 4–5.5 and 3.0–4.5 mg/mL, respectively, while *Bacillus* species and *S. epidermidis* were the most sensitive strains. The remaining ext. showed no or lower effects.	(Nenaah & Ahmed, 2015)
Roots, stems	India	EtOH ext.	*In vitro:* ADM	Up to 10 mg/mL medium	G–ve: *Chlamydia pneumoniae* (MTCC 7162), *P. aeruginosa* (MTCC 10462), *S. typhi* (MTCC 3231). G + ve: *B. anthracis* (MTCC 10095), *Bacillus thurengenesis* (MTCC 10484).	Root ext. showed MIC of 1.2–2.5 mg/mL, *B. anthracis* being most susceptible. Stem ext. showed MIC of 1.3–8.9 mg/mL, and *P. aeruginosa* was most sensitive to the stem ext.	(Panchal & Singh, 2015)
Stem, fruits, Leaves, flowers	Saudi Arabia	70% MeOH ext. and its Hex, Ether, CHL and Aq. fractions	*In vitro:* modified method (spotting on agar plates)	100 µg/10 µL/spot	G–ve: *K. pneumoniae, E. coli, P. vulgaris, P. aeruginosa* G + ve: *B. subtilis, S. aureus*	All fractions were active only against *K. pneumoniae* and produced ZOI of 10–24 mm. Nonpolar fractions were more potent, while ether Fct. was the most potent.	(Morsy et al., 2016)
Leaves, flowers	Rajasthan, India	95% MeOH ext.	*In vitro:* Growth kinetic study	1/100 in medium	G–ve: carbapenem-sensitive *Acinetobacter baumanii* (ATCC 19606) and carbapenem-resistant *A. baumanii* RS 307	Significant growth inhibition was observed. Synergistic effects with imipenem against both tested bacteria were observed.	(Tiwari et al., 2016)
Leaves	Saudi Arabia	MeOH ext.	*In vitro*: TDM	Serial dilution: 0.15–75 mg/mL	G–ve: *P. aeruginosa, P. vulgaris, S. typhi, Enterobacter cloacae, K. pneumoniae, E. coli.* G + ve: *S. aureus, S. pneumoniae, B. cereus, E. faecalis.*	It showed moderate activity with MIC of 9.4–37.5 mg/mL. Lowest MIC was against the G–ve (*E. coli, E. cloacae, E. faecalis* and *K. pneumoniae*).	(Al-Ghanayem et al., 2017)
Leaves	NR	80% EtOH, n-Hex and Cold and Hot water ext.	*In vitro*: WDM,	25 mg/well	Vancomycin and methicillin resistant isolates from wounds: G + ve: *S. aureus*, *P. mirabilis.* G–ve: *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. pyogenes*	EtOH ext. was most potent and showed ZOI of 8.03–16.03 mm. *S. aureus* was the most susceptible bacterium.	(Akindele et al., 2017)
Leaves	Saudi Arabia	MeOH ext.	*In vitro*: DDM	50 µL/disc	G + ve: MRSA	Significantly inhibited growth of MRSA and produced ZOI of 18 mm.	(Alzahrani et al., 2017)

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Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Leaves	India	MeOH ext.	*In vitro*: DDM	500 µg/disk	G–ve: *E. coli, K. pneumonia, S. pyogenes, P. aeruginosa* G + ve*: S. aureus*	Produced ZOI of 12 ± 1–31.8 ± 1.58 mm. *S. pyogenes* and *P. aeruginosa* were the most and least susceptible bacteria, respectively.	(Jeya & Veerapapgu, 2017)
Crude CPL, Serum of CPL	Egypt	Crude CPL, CPL serum, and MeOH, EtOH, CHL, and Aq. ext. of dried CPL serum	*In vitro*: WDM	Crude CPL and CPL serum: 5–30 µg/ wells. Different ext. 2.5–12.5 mg/ wells	G–ve: *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027). G + ve: *S. aureus* (ATCC 6538P), *B. subtilis* (ATCC 6633)	Crude CPL was active only against G + ve with ZOI of 7.6 ± 2.1–8.5 ± 1.0 mm and MIC of 250 µg/mL. CPL serum was active on all tested strains with ZOI of 13.3 ± 1.9–20.3 ± 1.5 mm and MIC of 200 µg/mL. EtOH and Aq. ext. showed highest activity with ZOI of 13.2 ± 0.6–19.6 ± 0.5 mm and MIC of 50 µg/mL. MeOH and CHL ext. showed ZOI of 11.6 ± 0.5–13.4 ± 1.2 and 10.3 ± 2.2–11.5 ± 0.6 mm, and MIC of 75 and 100 µg/ mL against G + ve, respectively.	(Hassan et al., 2017)
Twelve endophytic fungi of leaf, stem and root	India	Crude ext. in DMSO	*In vitro*: WDM	60 µL/well	Five pathogenic bacteria: G–ve: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700,603 G + ve: *B.* subtilis, *S. epidermidis*	Among 12 endophytes extracts of five of them (*F. solani*, *Cladosporium herbarum*, *Curvularia pallescens*, *A. alternata* and *Drechslera nodulosa*) showed broad antibacterial effects. Produced ZOI was in range of 9–32 mm, the largest was for both *Cladosporium herbarum* and *F. solani* against *E. coli* and *S. epidermidis,* respectively. The smallest ZOI was for *Drechslera nodulosa* against *B. subtilis.* *Curvularia pallescens* and *A. alternata* were the most potent endophytes against tested bacteria.	(Aharwal et al., 2014)
35 fungal endophytes of leaves	Brazil	8 mm disc of fungal colony	*In vitro*: agar block method	8 mm disc cut from fungal colony	G–ve: *E. coli* UFPEDA 224, *E. aerogenes* UFPEDA 739, *S. typhi* UFPEDA 478, *P. aeruginosa* UFPEDA 735, *P. vulgaris* UFPEDA 740. G + ve: *S. aureus* UFPEDA 02*, B. subtilis*, *E. faecalis* UFPEDA 86, UFPEDA 138, *S. pyogenes* UFPEDA 07, *Mycobacterium smegmatis* UFPEDA 71	Six endophytic fungi showed antimicrobial effects on some of tested microorganisms. ZOI was 12–20 mm. *C. pallescens* (URM 6048) showed ZOI of > 15 mm against *S. aureus* and *S. pyogenes.* *Cladosporium cladosporioides* (URM 6084) and *Xylaria* sp. (URM 6085) produced ZOI 00 against the alcohol-acid resistant bacterium *M. smegmatis.* Endophytes of old leaves showed better activity.	(Nascimento et al., 2015)

Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Twenty endophytic fungi	India	EtAc ext of 20 endophytes	*In vitro*: WDM, MIC: MDM, TKA	4 mg/well, serial dilutions of 50 mg/mL, 0.5, 1 and 2 MIC	G–ve: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Serratia marcescens* ATCC 27137, *S. flexneri* ATCC 12022, *S. typhi* ATCC 13311, *P. mirabilis* ATCC 43071, *K. pneumoniae* ATCC 700,603 G + ve: *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 259323)	Active on all tested bacteria with ZOI of 8–17.33 mm. Extracts of seven endophytes were active on all tested bacteria. Maximum ZOI (17.33 mm) was produced by extract of both *Aspergillus nomius* and *A. oryzae* against *S. typhi*, and extracts of *F. solani*, and *Curvularia hawaiiensis* against *S. flexneri* and *S. marcescens*, respectively. *S. aureus* was sensitive to extracts of all 20 endophytes, while *E. coli* was least sensitive strain. MIC were in range of 15.6 to 250 µg/well. TKA on *S. typhi* revealed bacteriostatic effects of the extracts on the tested bacterium.	(Rani et al., 2017)
Stems, leaves, roots	Pakistan	80% MeOH ext. and its Hex, CHL and EtAc Fct.	*In vitro:* MDM	2.5–40 mg/mL medium	Rifampicin-sensitive *Mycobacterium tuberculosis* (H37Rv), Rifampicin-resistant *M. tuberculosis* (TMC331)	Hex. Fct. of stem and leaves showed MIC of 10 and 20 mg/mL on both of the tested strains, respectively. CHL Fct. of stem showed MIC of 20 and 40 mg/mL, respectively. EtAc Fct. of stem and Hex Fct. of root showed respectively, MIC of 20 and 40 mg/mL against H37Rv. The crude MeOH ext. and its other remaining fractions did not show any effects.	(Ullah et al., 2017)
Leaves	India	PetE, CHL and EtOH ext.	*In vitro*: WDM	3.125–13.5 mg/well	G–ve: *E. coli, K. pneumoniae, P. aeruginosa* G + ve: *S. aureus*	EtOH ext. was more potent with ZOI of 11–18 mm at the lowest dose. *E. coli* and *S. aureus* were respectively the most and the least susceptible strains. Remaining extracts showed milder activity.	(Ul-Zaman & Ahmad, 2017)
Leaves	Nigeria	EtOH, MeOH and Aq. ext.	*In vitro*: WDM	3.125–25 mg/well	G–ve: *E. coli*, *P. aeruginosa*, *S. typhi* G + ve: *S. aureus*	The extracts showed ZOIs of 7.0–23.0 mm and MIC of 125–500 mg/mL. EtOH ext. was the most potent sample. *S. aureus* was the most susceptible bacterium.	(Akin-Osanaiye & Okhomina, 2018)
Leaves	Jazan, Saudi Arabia	EtOH ext.	*In vitro*: WDM	4–13 µg/mL	G–ve: *E. coli, S. pyogenes* G + ve*: S. aureus, B. subtilis*	It produced ZOI of 5–21 mm at the highest dose. *E. coli* and *S. aureus* were the most susceptible bacteria.	(Alhazmi et al., 2018)
Stem barks, leaves, roots	Ethiopia	EtOH, MeOH, Hex, and Aq. ext.	*In vitro:* DDM	Disc soaked with 100 µL of 30–60 mg/mL	G–ve: *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922). G + ve: *S. aureus* (ATCC 29223)	EtOH ext. was the most potent followed by MeOH, Hex and Aqueous ext. EtOH ext. produced ZOI of 12.70–24.50 mm, while *S. aureus* was the most susceptible bacterium.	(Asfere et al., 2018)

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Table 2 (*continued*)

Plant parts	Geographical origin	Extracts/fractions	Test model	Dosage	Test microorganisms	Results	References
Flowers, leaves, roots	India	EtOH, MeOH and CHL ext.	*In vitro:* DDM	Disc soaked with 25–100 µg/mL ext.	G–ve: *E. coli* G + ve: *B. subtilis, S. aureus*	EtOH, MeOH and CHL ext. of leaf showed ZOI of 11 and 20, 18 and 20, 14 and 17 mm, respectively against *E. coli* and *S. aureus*. EtOH, MeOH and CHL ext. of flower produced ZOI of 18 and 17, 20 and 19, 16 and 17 mm, respectively against *E. coli* and *S. aureus*. EtOH, MeOH and CHL ext. of root showed ZOI of 20 and 21, 19 and 20, 17 and 16 mm, respectively against *E. coli* and *S. aureus*. *B. subtilis* was not susceptible to the extracts.	(Kar et al., 2018)
Leaves	Iraq	70% EtOH ext.	*In vitro:* DDM	36 µg/disc	G–ve: *E. coli*, *Pseudomonas* sp. G + ve: *B. subtilis, S. aureus*	It produced ZOIs of 17 and 18 mm and MIC of 31.25 and 250 µg/mL against *B. subtilis* and *S. aureus*. The G–ve bacteria showed mild susceptibility.	(Naser et al., 2019)
Shoots, roots	Egypt	Aq. ext.	*In vitro:* DDM	Discs soaked in 1–10% (w/v) ext. in water	G–ve: *E. coli* (MTCC 118) G + ve: *S. aureus* (MTCC 96)	Dose-dependent activity was observed. It produced ZOI of 9–21 and 7–19 mm, respectively against *E. coli* and *S. aureus*.	(Radwan et al., 2019)
Leaves	India	Aq, MeOH and PetE ext.	*In vitro*: WDM & MTD	2–4 mg/well. SD of 100 mg/mL (for MIC)	G–ve: *P. aeruginosa* (ATCC 27853), *S. marcescens* (ATCC 27137), *S. flexneri* (ATCC 12022), *S. typhi* (ATCC 13311), *E. coli* (ATCC 25922), *P. mirabilis* (ATCC 43071), *K. pneumonia* (ATCC 700603). G + ve: *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 259323).	Aq ext. was most active on all tested bacteria with ZOI of 10.33–13.66 mm, and MIC 1.25–2.5 mg/mL. MeOH and PetE ext. were active on some of tested strains. *S. aureus* was only susceptible to Aq ext. and showed highest ZOI and lowest MIC (1.25 mg/mL).	(Rani et al., 2019a)
Leaves	Pakistan	MeOH ext.	*In vitro:* DDM	10 µg/disc	G–ve: *P. mirabilis, P. aeruginosa* G + ve: *B. cereus*	It produced ZOI of 16 ± 2–19 ± 2 mm against tested strains.	(Bilal et al., 2020)

Abbreviation: ADM, agar dilution method; ACT, acetone; Aq., Aqueous; BtOH, butanol; CHL, chloroform; DDM, disc diffusion method; DiEE, diethyl ether; Eq., equivalent; EtAc, ethyl acetate; EtOH, ethanol; ext., extract; Fct., fraction; Flv., flavonoids; 2fd, two-fold dilution; MDM, micro-dilution method; MeOH, methanol; MIC, minimum inhibitory concentration; NF, not found; PetE, petroleum ether; RM, raw materials; SD, serial dilution; TDM, tube dilution method; TKA, time-kill assay; WDM, well diffusion method; ZOI, zone of inhibition.

Table 3 Antifungal activity of *C. procera* latex and different extracts.

Plant parts	Geographical origin	Extracts/Fractions	Test model	Dosage	Test microorganisms	Results	References
CPL	Ethiopia	Aq. ext. and PetE, CHL, MeOH Fct. of 80% EtOH ext.	*In vitro*: WDM	200 µg/well	*Candida albicans* (ATCC 10231)	The Aq. ext. was more potent. Thus, presumably the potency could be related to the more polar compounds of CPL.	(Desta, 1993)
Leaves	United Arab Emirates	95% EtOH ext.	*In vitro:* ADM	NR	*C. albicans*	It showed MIC of 2 mg/mL.	(Tanira et al., 1994)
CPL	India	PetE, MeOH and Aq. ext.	*In vitro*: WDM	2 mg/well	*C. albicans*	PetE, MeOH and Aq. extracts produced ZOI of 18 ± 1.6, 17.5 ± 0.6, 7.05 ± 1, and MIC of 128, 128 and 1600 mg/mL, respectively.	(Sehgal et al., 2005)
Leaves, stem barks, roots	Nigeria	Aq., 50% EtOH ext. and Hex, PetE, and CHL Fct. of 50% EtOH ext.	*In vitro:* ADM	2.5–20 mg/mL media	*Aspergillus niger, Trichophyton rubrum,* and *Microsporum gypseum*	Aq. ext. of all parts showed the most potent effects and significantly inhibited (97.80%) growth of tested fungi.	(Hassan et al., 2006)
Leaves, stems	Nigeria	50% EtOH ext. and its CHL, EtAc and BtOH Fct.	*In vitro*: WDM	Ext. 20 and 40 mg/mL. Fct. 5 mg/mL	*C. albicans*	MeOH ext. of leaves and stems (20 mg/mL) showed ZOI of 7 and 7.5 mm, respectively. Fct. of leaf and stem (5 mg/mL) produced ZOI of 7.5 and 8 mm, respectively.	(Oladimeji et al., 2006)
Leaves, CPL	Nigeria	60% EtOH ext.	*In vitro*: WDM	50 µL/well (ca. 5 mg RM /well	*A. niger, Aspergillus flavus, C. albicans*, and *Microsporium boulardii*	Ext. of CPL and leaves showed ZOIs of 2.5– 8.5 and 1.2–4.6 mm, and MIC of 5.0–12.5 and 7.5–17.5 mg/mL, respectively. The most susceptible fungus to the extracts was *A. niger*.	(Kareem et al., 2008)
Stem barks	Nigeria	Aq. ext.	*In vitro*: DDM, TDM	Disk soaked in 4 mg/mL. 0.5–5.0 mg/mL	*Epidermophyton floccosum, Tricophyton gypseum*	It produced ZOI of 10 and 8 mm and MIC of 0.5 and 0.9 mg/mL, respectively against tested fungi.	(Kuta, 2008)
Leaves and flowers	Pakistan	80% EtOH, 80% MeOH, and 80% ACT ext.	*In vitro*: DDM, MDM	1.5 mg/disc	*A. niger, Fusarium oxysporum*	EtOH Leaf ext. showed ZOI of 5.3 mm (MIC: 35 mg/mL) and 7.7 mm (MIC: 33 mg/mL) against respective tested fungi. EtOH Flower ext. showed ZOI of 3.9 and 5.8 mm, respectively. The MeOH and ACT extracts produced milder effects.	(Ahmad et al., 2011)
Leaves	India	PetE, CHL, EtAc, EtOH ext.	*In vitro:* DDM	Discs saturated with 6 mg/mL solution	*Microsporum canis* (MTCC 3270), *Microsporum fulvum* (MTCC 7675), *Trichophyton mentagrophytes* (MTCC 7250)	The EtOH ext. being the potent sample produced ZOI of 12.5, 12.5 and 9.13 mm, respectively against tested fungi.	(Verma et al., 2011)
Aerial parts	India	MeOH ext.	*In vitro*: WDM	5 mg/well	*A. niger* (MTCC 2723), *Penicillium expansum* (MTCC 2006), *F. oxysporum* (MTCC 1755).	Moderate effects with ZOI of 9–11 mm and MIC of 100–152 mg/mL. *F. oxyporum* was most sensitive.	(Vadlapudi et al., 2012)
Leaves	India	Hex, EtAc and MeOH ext.	*In vitro*: WDM	10–100 µg/well	*Fusarium* sp.	The EtAc ext. produced ZOI of 15.10 ± 2.86 mm.	(Velmurugan et al., 2012)

(*continued on next page*)

Table 3 (*continued*)

Plant parts	Geographical origin	Extracts/Fractions	Test model	Dosage	Test microorganisms	Results	References
Leaves	India	EtAc, MeOH and CHL ext.	*In vitro*: WDM	50–250 mg/mL	*A. flavus, T. rubrum, Trichophyton tonsurans, T. mentagrophytes, E. floccosum.*	EtAc and CHL ext. produced moderate effects with ZOI of 4–11 against some tested fungi. *T. mentagrophytes, E. floccosum* and *A. flavus* showed the largest ZOI to EtAc ext. while MeOH ext. did not show activity.	(Halu & Vidyasagar, 2012)
Leaves	Agra, India	EtOH ext. and its CHL: MeOH (5:1) Fct.	*In vitro:* DDM	Discs soaked with 600 µg/mL ext.	Five isolates: *M. canis* (MTCC 3270), *M. fulvum* (MTCC 7675), *T. mentagrophytes* (MTCC 7250), *A. niger* (MTCC 2587), *Aspegillus fumigatus* (MTCC 8636).	Crude EtOH ext. showed ZOI of 11.5 ± 0.025–17.5 ± 0.025 mm. *A. fumigatus* was resistant to the crude ext. The fractions produced ZOI of 10.5 ± 0.025 to 19.0 ± 0.035 mm, while *T. mentagrophytes* and *M. canis* were the most sensitive tested fungi.	(Verma et al., 2012)
Leaves:	Saudi Arabia	80% MeOH ext. and Flv. fraction	*In vitro:* WDM, TDM	40 µg/well	*C. albicans* (ATCC 10231), *Candida tropicalis*, *Saccharomyces cerevisiae* (ATCC 10716), *A. niger*, *A. flavus*, and *Penicillium chrysogenum*	Crude ext. showed ZOI of 12.0 to 22.5 mm, and MIC of 0.08–0.32 mg/mL. Flv. Fraction produced ZOI of 18–30 mm and MIC of 0.04–0.32 mg/mL.	(Nenaah, 2013a)
Stem	Nigeria	Aq. ext. (decoction)	*In vitro*: ADM	1 mL/3 mL media	*Microsporum* sp., *Trichophyton* sp.	It caused complete growth inhibition of the isolated dermatophytes within 10 days incubation period.	(James et al., 2013)
CPL	Egypt	Crude CPL serum	*In vitro:* WDM	300 µL/well	*T. rubrum* (AUMC 1804), *C. albicans* (AUMC 3880), *Aspergillus terreus*	It produced ZOIs of 11.10–21.8 mm. *C. albicans* was most susceptible.	(Mohamed et al., 2014)
CPL	Nigeria	Fresh CPL	*In vitro*: ADM (modified)	20, 50, and 100% CPL and (1:3) in medium	*Trichophyton* sp., *Microsporum* sp., *Epidermophyton* sp.	Broad and dose dependent antifungal effects. *Trichophyton* sp. was more susceptible and showed only 39.7, 45.8- and 51.06-mm growth spread on day 6th, against 100, 50 and 20% CPL, respectively.	(Aliyu et al., 2015)
35 fungal endophytes of leaves	Brazil	8 mm disc of fungal colony	*In vitro*: agar block method	8 mm disc cut from fungal colony	Five human pathogenic fungi: *C. albicans* URM 5889, *Malassezia furfur* URM 4849, *E. floccosum* URM 5110, *Trichosporum cutanum* URM 5743, *Fusarium solani* URM 5776. Two phytopathogens: *Colletotrichum dematium* URM 3315, *F. oxysporum* URM 5283	Only six endophytes were active against some of the tested human pathogens, and one plant pathogen. *C. pallescens* produced ZOI > 15 mm against *C. dematium*. *C. cladosporioides* inhibited *E. floccosum*. Some strains were resistant to all of the endophytic fungi. Endophytes isolated from old leaves showed better activity.	(Nascimento et al., 2015)
Leaves, flowers, CPL	Saudi Arabia	Aq., 80% MeOH, DiEE ext.	*In vitro*: DDM	20 µL/disc	Yeasts: *S. cerevisiae, C. albicans, C. tropicalis* Mycelial fungi: *A. niger, A. flavus, P. chrysogenum*	Yeasts were more susceptible (ZOI: 9.5–26.5 mm) than the mycelial fungi (ZOI: 9.0–20.5 mm). The MeOH ext. was most effective (ZOI of 15.0–26.5 mm, MICs: 0.25–1.5 mg/mL).	(Nenaah & Ahmed, 2015)
Roots, stems	India	EtOH ext.	*In vitro*: ADM	Up to 20 mg/mL	*A. fumigatus, Blastomyces dermatitidis, C. albicans, Candida neoformans, Candida vaginitis*	MIC of root ext. was 12.2 ± 0.015–14.5 ± 0.016 mg/mL. MIC of stem ext. was 10.5 ± 0.013–13.3 ± 0.015 mg/mL. *C. neoformans* and *C. vaginitis* were most susceptible to both extracts.	(Panchal & Singh, 2015)

Table 3 (*continued*)

Plant parts	Geographical origin	Extracts/Fractions	Test model	Dosage	Test microorganisms	Results	References
Leaves	India	Aq. MeOH, EtOH, & ACT ext.	*In vitro:* Food poison method and MDM	Eq. 30 mg DRM/mL media, and 0.39–200 mg/mL media	*Alternaria alternata* (4 isolates A1-A4)	EtOH ext. completely inhibited fungal growth. MIC and MFC of EtOH ext. were 6.25 and 12.5 mg/mL, respectively. MeOH and ACT extracts caused 76–81 and 86–91% growth inhibition. Aq. ext. was very weak.	(Srivastava & Singh, 2015)
Stems, fruits, leaves, flowers	Saudi Arabia	70% MeOH ext. and its Hex, Ether, CHL and Aq. Fct.	*In vitro:* modified ADM (spotting)	100 µg/10 µL/spot	*A. niger*	Very mild activity was observed. Only hexane and ether fractions of stem and fruits produced ZOI of 3 mm.	(Morsy et al., 2016)
CPL	Egypt	Crude CPL, CPL serum and MeOH, EtOH, CHL, and Aq. ext. of CPL dried serum	*In vitro*: WDM	CPL and serum: 5–30 µg/wells. CPL serum ext.: 2.5–12.5 µg/wells	*C. albicans* (ATCC 10231), *A. niger* (ATCC 16404).	Crude CPL and CHL extract were not active. CPL serum produced ZOI of 10.5 ± 0.7–12.3 ± 0.7 mm and MIC 200 µg/mL. EtOH and Aq. ext. produced ZOI of 12.2 ± 1.1–16.3 ± 0.7 mm and MIC 50–75 µg/mL. The *C. albicans* and *A. niger* were susceptible to EtOH and Aq. extracts, respectively. MeOH ext. produced ZOI of 12.2 ± 1.5 mm and MIC 75 µg/mL only against *C. albicans*.	(Hassan et al., 2017)

Abbreviation: ADM, agar dilution method; ACT, acetone; Aq., Aqueous; BtOH, butanol; CHL, chloroform; DDM, disc diffusion method; DiEE, diethyl ether; DRM, dried raw material; Eq., equivalent; EtAc, ethyl acetate; EtOH, ethanol; ext., extract; Fct., fraction; Flv., flavonoids; MDM, micro-dilution method; MeOH, methanol; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concentration; NF, not found; PetE, petroleum ether; RM, raw materials; TDM, tube dilution method; WDM, well diffusion method; ZOI, zone of inhibition.

Table 4 Antiprotozoal activity of *C. procera* different extracts.

Plant parts	Geographical origin	Extracts/ fractions	Test model	Dosage	Test microorganisms	Results	References
Whole plant	India	50% EtOH ext.	*In vitro:* On infected erythrocytes of animal	100 µg/ mL	*Plasmodium berghei* NK 65	The ext. produced 36.57 ± 9.49% inhibition of *P. berghei in vitro*, but failed to show activity in the experimental animal (*Mastomys natalensis*).	(Misra et al., 1991)
Buds, roots, flowers	India	EtOH ext. and its EtAc, ACT and MeOH Fct.	*In vitro:* Human erythrocytes	Serial dilutions: 0.0625–2 mg/mL	Chloroquine-sensitive (MRC20) *Plasmodium falciparum.* Chloroquine-resistant (MRC76) *P. falciparum*	The ext. showed IC_{50} of 0.1–1 and 0.3 to 0.9 mg/mL against MRC20 and MRC76, respectively. Fractions at doses 62.5–125 µg/mL, caused 7.5–61.38% inhibition of MRC20 and 3.4–41.08% inhibition of MRC76. Root fractions were most active on both strains.	(Sharma & Sharma, 1999)
Leaves, stems, roots, flowers, buds	India	EtOH ext.	*In vitro*	0.062.5–2 mg/mL	Chloroquine-sensitive *P. falciparum* (QS-PF), chloroquine-resistant *P. falciparum* (QR-PF).	At lower doses produced IC_{50} of µg/ mL. IC_{50} of all parts ext. against QS-PF and QR-PF were 0.11–0.47 and 0.52–1.22 µg/mL, respectively. The flowers and buds' extracts were the most potent samples.	(Sharma & Sharma, 2000)
Flower	India	EtOH ext.	*In vitro*	12.5–100 µg/ mL	Chloroquine-sensitive *P. falciparum.*	It showed a dose-dependent *in vitro* antiplasmodial effect and caused 17–67% inhibition of *P. falciparum.*	(Simonsen et al., 2001)
Leaves	Nigeria	EtOH ext. and its different Fct.	*In vitro:* Human erythrocytes	1–5 mg/ mL	*P. falciparum* in patients' erythrocytes	The MeOH and aqueous fractions at dose 5 mg/mL produced 57.1 and 53.6% elimination of the parasites, respectively.	(Mudi & Bukar, 2011)
Flower	Kenya	MeOH	*In vitro:* MDM	1.56–100 mg/ mL	Chloroquine-sensitive *P. falciparum.* chloroquine-resistant *P. falciparum.*	Strong activity with IC_{50} < 25 µg/ mL against the resistant strain.	(Muthaura et al., 2015)
Leaves	India	EtOH ext.	*Ex vivo:* human RBC culture	NR	Chloroquine-sensitive (P*f*3D7) *P. falciparum.* chloroquine-resistant (P*f*INDO) *P. falciparum*	Showed IC_{50} of 2.5 and 2.9 µg/mL, respectively against the sensitive and resistant tested strains.	(Singh et al., 2015)
Leaves	Saudi Arabia	MeOH ext. and its PetE, CHL, EtAc, BtOH and Aq. Fct.	*In vitro*	12.5–100 µg/ mL	*Leishmania major*	The crude MeOH extract showed dose-dependent effects i.e., 52.6 to 35.5% parasite inhibition and IC_{50} of 66.8 µg/mL. Amongst fractions, the CHL, EtAc and Aq. fractions showed more potent effects with IC_{50} of 44.2, 33.5 and 26.3 µg/mL, respectively.	(Nasr, 2020)

Abbreviation: ACT, acetone; Aq., Aqueous; BtOH, butanol; CHL, chloroform; EtAc, ethyl acetate; EtOH, ethanol; ext., extract; Fct., fraction; MDM, micro-dilution method; MeOH, methanol; PetE, petroleum ether.

In addition, *C. procera* grows in all types of soils including roadsides and soils polluted with heavy metals. Since the plant has a high capacity of absorbing various chemicals elements (e.g., heavy metals), it bioaccumulates higher concentrations of hazardous heavy metals such as Cr, Cd, Ni, Pb, etc. and other environmental pollutants in into its different organs/-parts. These accumulated heavy metals further contribute to the toxicity of the plant (Naz et al., 2020).

CPL bearing pH 5.2, has a caustic effect on mucosal membranes of the body, while cardiac glycosides of *C. procera*, sim-

Table 5 Antiviral activity of *C. procera* latex and different extracts.

Plant parts	Geographical origin	Extracts/ fractions	Test model	Dosage	Test microorganisms	Results	References
CPL	India	Aqueous dilutions	*In vitro:* Applied on leaf surface	0.1, 1 and 10% in water	Tobacco mosaic virus (TMV)	All dilutions significantly (80%) inhibited growth of TMV.	(Khurana & Singh, 1972)
Leaves	India	Hot Aq. ext.	p24 antigen assay	2–5 mg/ well	Human immunodeficiency virus (HIV-1)	A potent and dose dependent anti-HIV effect was observed, and at highest dose elicited 60 ± 1.3% inhibition of HIV p24 antigen expression.	(Mohanraj et al., 2010)
Leaves	India	Hex, EtAc, and MeOH ext.	*In vitro:* incubation *In vivo*: injected to *Penaeus monodon*	10 µL of 5 mg/mL + 5 µL viral Susp. 10 µL, IM to shrimp	White spot syndrome virus (WSSV)	The EtAc extract effectively suppressed growth of WSSV during the incubation, and in *in vivo* study, caused 80% survival of the experimentally-infected *P. monodon*. The Hex and MeOH extracts showed lower effects.	(Velmurugan et al., 2012)
Leaves, root barks, flowers	Pakistan	Aq. and MeOH ext.	*In vitro*: Cell culture technique	2fd: 0.032–5 mg/mL	Foot and mouth disease virus (FMDV) a member of *Apthovirus* spp.	The MeOH ext. of leaf showed maximum effects. MeOH leaf ext. and Aq. rootbark ext. at 0.15–0.625 mg/mL showed antiviral effects without cytotoxicity. Aq. ext. of flower showed activity at 0.075–0.15 mg/mL, without cytotoxicity. MeOH flower ext. at 0.15 mg/mL showed activity, while the Aq. leaf ext. was not active.	(Saher et al., 2018)

Abbreviation: ACT, acetone; Aq., Aqueous; EtAc, ethyl acetate; ext., extract; Fct., fraction; 2fd, two-fold dilution; IM, intramuscular; MeOH, methanol; Susp., suspension.

ilar to those of *Digitalis*, coarsely increase heartbeat and subsequently, cause death of the animals (Al-Mezaine et al., 2005, 2008).

It is worth noting that CPL caused ocular toxicity while being splashed into human eyes, as several cases in this regard have been documented. For instance, in India (where the plant is worshiped), CPL splashed into the eyes caused ocular toxicity in terms of ocular inflammations, corneal oedema, dimness of vision that might be associated with keratouveitis (Basak et al., 2009; Lakhtakia et al., 2010). Similarly, some cases of permanent endothelial cell injury due to contact and intracorneal penetration of CPL into eyes of some people in Saudi Arabia have been reported (Al-Mezaine et al., 2005, 2008). However, owing to its local anesthetic effect on corneal epithelial cells, it is not very painful when CPL is splashed into the eye. Interestingly, CPL is not very toxic to the corneal epithelium, but it is highly toxic to the corneal endothelial cells, causing serious hazards in terms of decrease in endothelial cells count and changes of their morphology (Al-Mezaine et al., 2005, 2008).

Toxicity of *C. procera* in experimental animals has also been reported. Arya & Kumar, (2004) reported pro-inflammatory effects of crude CPL and its methanolic extract in experimental animals after being injected with 0.1 mL aqueous solution of the tested samples through sub-plantar injection. Both the dried CPL and its extract revealed inflammatory effects on the paw of animals with a rapid onset and peak effect within the first 2 h following injection. Jato et al., (2010) reported a dose-dependent toxicity of oral administration of aqueous *C. procera* leaf extracts in rabbits. Administration of CPL and ethanolic *C. procera* leaf extract caused significant elevation in level of heart enzymes e.g., creatine kinase-MB isoenzyme (CK-MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in serum along with impairment of the normal structure of heart associated with inflammation and necrosis of cardiac myocytes in experimental animals. Meanwhile, an increase in the malondialdehyde (MDA) level in treated animals' serum was observed. MDA is an index of lipid peroxidation or production of reactive oxygen species (ROS). However, it was found that the toxic effects of CPL and ethanolic *C. procera* leaf extract was dose-dependent (Ahmed et al., 2016). Likewise, nephrotoxicity of *C. procera* fresh leaves (Mahmoud et al., 1979b), CPL and ethanolic *C. procera* leaf extract has been reported through *in vivo* study (Fahim et al., 2016).

Interestingly, in another *in vivo* study it was found that CPL toxicity was related to the rubber (>90% in crude CPL) portion and other organic fractions of the latex. The rubber-free or purified water-soluble proteins of CPL was nontoxic to the animals even at high oral dose of 5000 mg/kg/bw (Bezerra et al., 2017).

C. procera wildly grows in many countries and is traditionally used for diverse medicinal purposes, as well. However, topical or external use of the plant seems to be somehow safer when compared to oral use of the plant. Meanwhile, caution is required to avoid direct contact of CPL and other herbal preparations of the plant with the eyes.

Overall, toxicity of *C. procera* local herbal preparations should not be ignored, and it is recommended to be cautiously

used as per advice of qualified traditional community healers or herbal medicine experts.

2. Antimicrobial activities of *C. procera*

Microbial diseases are caused by any of the four common types of microorganisms which include bacteria, fungi, protozoa and viruses. Many MPs including *C. procera* have been traditionally used as natural antimicrobial remedies in treatment of various infections caused by the pathogenic microorganisms (Joshi, 2018). As per the literature survey, *in vitro* antimicrobial potentials of *C. procera* have been investigated against a wide range of microorganisms, which are discussed below under four sub-headings specified for antibacterial, antifungal, anti-plasmodial, and antiviral activities of *C. procera*. For ease of understanding and reading, the data are shown in tabulated forms that includes the used plant parts, geographical origin, solvents and extracts' types, test model, dosage, test microorganisms along with their ATCC or NTCC numbers, the brief results and references.

2.1. Antibacterial activity of C. procera latex and extracts/fractions of different parts

The CPL and different parts of *C. procera* collected from several countries and geographical origins have been investigated for antibacterial properties against numerous Gram-negative (G + ve) and Gram-positive (G–ve) bacterial strains (Mascolo et al., 1988; Yesmin et al., 2008; Nenaah, 2013a; Tiwari et al., 2016; Radwan et al., 2019). About 57 original publications on antibacterial effects of *C. procera* were reviewed and their summarised results are presented in tabular form in Table 2.

As shown in Table 2, different parts/products of *C. procera* e.g., arial parts, buds, flowers, fruits, latex (CPL), leaves, roots, root-bark, seeds, stems/twigs, stem-barks and whole plant have been tested *in vitro* against over 65 different bacterial strains. The dominant plant parts that have been tested for antibacterial activity (study case) were the leaves (37 study cases), followed by flowers (14 cases), roots (11 cases), stems (9 cases), CPL (8 cases), fruits (3 cases), stem-barks (3 cases), and the remaining parts with only one study case for each. In few cases, extracts prepared from *C. procera* endophytes also showed antibacterial properties (Aharwal et al., 2014; Rani et al., 2017). Wells' diffusion method (WDM) and disc diffusion method (DDM) were the two commonly used antibacterial assay methods, while Micro-dilution method (MDM), tube dilution method (TDM) and time kill assay (TKA) were also used by some authors. Different authors used various solvents extracts and varied dose ranges (0.01–25 mg/well) in *in vitro* antibacterial assay, and consequently diverse levels of antibacterial potency of *C. procera* different parts and CPL were reported. For instance, in some studies, aqueous extract of *C. procera* showed potent antibacterial effects (Yesmin et al., 2008; Samy & Chow, 2012; Panda, 2014), while in another study the aqueous extract of *C. procera* was the least active sample (Parabia et al., 2008; Asfere et al., 2018). Some authors reported methanolic and ethanolic extracts of the plant with good potency (Salem et al., 2014; Kar et al., 2018), while others reported nonpolar fractions of other organic solvents extracts of *C. procera* as potent samples (Morsy et al., 2016). In certain cases, the EtAc extracts were the most potent samples (Mohanraj et al., 2010). Similarly, some authors reported CPL extracts with potent antibacterial effects (Kareem et al., 2008), while in another study, CPL did not show antibacterial effects (Jain et al., 1996). Unfortunately, some authors did not clearly mention the doses used in WDM or DDM of *in vitro* antibacterial assay (Adamu et al., 2005; Oladimeji et al., 2006; Mainasara et al., 2011, 2012).

Overall, due to experimental inconsistencies, comparison of the results of *C. procera* antibacterial studies seems to be very complicated. However, more systematic and in-dept studies are encouraged to explore antibacterial potential of *C. procera* isolated compounds and their MOA that hopefully serve as new antibacterial agent (s).

2.2. Antifungal activity of C. procera latex and extracts of different parts

In vitro antifungal activity of CPL and extracts of different parts from *C. procera* against numerous fungi and yeasts have been evaluated by several researchers. As per about 23 reviewed literatures, different extracts of *C. procera* and of its CPL have been tested for *in vitro* antifungal potential against different fungi and yeasts. In this regard, for ease of reading the summarized data extracted from reviewed literatures are presented in tabular forms in Table 3.

As shown in Table 3, CPL and extracts of different parts e.g., arial parts, flowers, fruits, leaves, roots, rootbarks, stems, and stem-barks of *C. procera* were reported for their *in vitro* antifungal effects against around 27 different fungi and yeasts. With regards to antifungal study of *C. procera*, its leaves were the dominant plant part (14 study cases), followed by CPL (7 cases), stems (4 cases), flowers (3 cases), roots (2 cases), stem-barks (2 cases), and only one study case for each of *C. procera* aerial parts and fruits. In a study, 35 fungal endophytes isolated from *C. procera* leaves were tested for their antifungal potential and six of them was active against some tested fungi (Nascimento et al., 2015).

Various authors used different solvent extracts and diverse dose ranges (0.005–5 mg/well) in *in vitro* antifungal assay of the test samples. Dissimilarities are obvious in the reported results of the studies. For example, aqueous extracts of *C. procera* leaves, stem barks and roots significantly inhibited (97.80%) the growth of tested fungi (Hassan et al., 2006). In another study, EtAc extract of leaves showed stronger antifungal effects when compared to other extracts (Halu & Vidyasagar, 2012), while CHL: MeOH (5:1) fraction of crude EtOH leaf extract of *C. procera* produced larger ZOI (up to 19 mm) when compared to that of the crude EtOH leaf extract (Verma et al., 2012). Nenaah & Ahmed, (2015) found that yeasts were more susceptible than mycelial fungi to both aqueous and MeOH extracts of *C. procera* leaves, flowers and CPL. However, in this study, MeOH extracts were more potent against the tested fungi (Nenaah & Ahmed, 2015). Interestingly, crude CPL at doses of 5–30 µg/wells was not active against *C. albicans* and *A. niger*, while EtOH and aqueous extracts of dried CPL serum 2.5–12.5 µg/wells elicited antifungal effects (ZOI up to 16 mm) against the tested fungi (Hassan et al., 2017).

Indeed, there are considerable controversy also in results of previously reported antifungal studies, and hence, comparison of the results of different works would be difficult.

2.3. Antiprotozoal activities C. procera

To justify the traditional uses of *C. procera* as anti-malarial remedy, some authors have investigated *in vitro* and *ex vivo* anti-plasmodial effects of the plant against *Plasmodium* species (Sharma & Sharma, 2000; Simonsen et al., 2001; Mudi & Bukar, 2011; Muthaura et al., 2015; Singh et al., 2015). In addition, antileishmanial property of *C. procera* has also been recently reported (Nasr, 2020). Table 4. depicts the antiprotozoal properties of *C. procera*.

Table 4 shows that eight published papers reported about the antiprotozoal properties of CPL and extracts of different parts of *C. procera*. Both flowers and leaves of *C. procera* were the dominant plant parts with 4 and 3 study cases, respectively, in antiplasmodial studies of the plant against different *Plasmodium* species. Buds, roots, whole plant and stems of *C. procera* were also studied against *Plasmodium* species but CPL is still not evaluated for anti-plasmodial or anti-protozoal potential. As shown in Table 4, *C. procera* leaves EtOH extract revealed a strong *ex vivo* anti-plasmodial effect with IC_{50} of 2.5 and 2.9 μg/mL against chloroquine-sensitive (P*f*3D7) and chloroquine-resistant (P*f*INDO) *Plasmodium falciparum*, respectively. In this study, *C. procera* was one the most potent plants among 22 medicinal plants used traditionally for treatment of malaria in Jharkhand, India (Singh et al., 2015).

Although *C. procera* has been traditionally used in treatment of cutaneous and digestive illnesses (Table 1), more recently, a dose-dependent *in vitro* antileishmanial potential of *C. procera* leaves was reported (Nasr, 2020). In this regard, studying antiprotozoal properties of *C. procera* against other protozoa particularly responsible for digestive illnesses would be interesting research topics. Considering traditional uses of *C. procera* in alleviating digestive system upsets, anthelminthic properties of this plant have already been established through *in vitro* (Shivkar & Kumar, 2003) and *in vivo* studies (Iqbal et al., 2005).

2.4. Antiviral activity of C. procera

Viral diseases are considered as one of the major threats for human, animals and plants globally. In addition to the challenges due to emergence of antiviral resistance and also side effects of available antiviral drugs (Bagla et al., 2012), the outbreaks of deadly viral diseases such COVID-19 which is severely challenging human survival worldwide further necessitates the discovery of vaccines or treatment solutions against these deadly microorganisms.

MPs have been proven to contain bioactive compounds with antiviral properties, and some of them have shown promising and broad spectrum antiviral potentials (Mukhtar et al., 2008; Mohanraj et al., 2010; Tariq et al., 2019). As per literature, *C. procera* has also been investigated for its *in vitro* and *in vivo* antiviral effects (Khurana & Singh, 1972; Mohanraj et al., 2010; Saher et al., 2018; Velmurugan et al., 2012), as summarised in Table 5.

Data in Table 5 indicates that the antiviral properties of CPL and other extracts of *C. procera* seem to be promising despite the limited studies that reported the antiviral potential of *C. procera* against only four viral species. Hence, further in-depth studies are required in order to isolate potent antiviral compounds from this miracle plant.

The overall data of Tables 2–5, show that in addition to the use of a wide dose-range in *iv-vitro* antimicrobial screening of *C. procera* (Mohanraj et al., 2010; Doshi et al., 2011; Ul-Zaman & Ahmad, 2017), a considerable inconsistencies were found in the results reported by different authors, and hence, it would be difficult to compare reported results of different works. For instance, some studies reported good antibacterial potential of the nonpolar fractions of *C. procera* extracts (Morsy et al., 2016), while some others reported methanolic extracts of the plant with higher *in vitro* antibacterial properties (Kar et al., 2018). However, such controversies in the results of biological screening of crude plant extracts could be attributed to several factors such as: geographical origin of raw materials, time of sample collection, nature or types of solvents used in the extraction, extraction procedures, purity of extracts, dose ranges, diversity in genetics of test microorganisms, assay methods, etc. (Muthaura et al., 2015).

Interestingly, from around 78 original research that had reported the antimicrobial properties of *C. procera* (see Tables 2–5), 35 (44.87%) of them have been conducted on raw materials collected from different parts of India, followed by Nigeria with 14 studies (17.94%), Saudi Arabia with 7 studies (8.97%), Pakistan with 7 studies (8.97%), Egypt with 6 studies (7.69%), Ethiopia with 2 studies (2.56%), and Bangladesh, Brazil, Kenya, Morocco, Iraq, United Arab Emirates and Yemen each with one study (1.28%).

Briefly, current review showed that, *C. procera* from about thirteen different countries have been collected and studied for different antimicrobial (antibacterial, antifungal, antiprotozoal and antiviral) activities by various groups of researchers. On the other hand, it was found that researchers had screened *C. procera* against different categories of microorganisms of human, animal and plant pathogens since 1980. Meanwhile, as per the overall data shown in Tables 2–5, to date *C. procera* is being screened *in vitro* against >90 different microbial strains including 34 G–ve bacteria, 31 G+ve and 2 g-variable pathogens, 27 fungal strains, 6 protozoa (including both chloroquine sensitive- and chloroquine-resistant *P. falciparum* and *Leishmania major*), and 4 viral pathogens, (see also Fig. 1).

However, thanks to all prior antimicrobial works of *C. procera* which besides justifying traditional uses of the plant in different infectious diseases, their compiled results also encourage further researches, and hence, more advanced investigations are now necessary in order to make use of this potent plant in drug discovery, particularly in development of antimicrobial formulations.

2.5. The use of C. procera as biomaterials in development of antimicrobial and wound healing approaches

Recently, CPL and different extracts of *C. procera* have been used as biomaterial in production of metallic nanoparticles and a bio-membrane that exhibited antimicrobial properties.

The silver nanoparticles (AgNPs) of 4–25 nm diameter that were developed from CPL serum showed potent *in vitro* antimicrobial effects when compared to the crude CPL. The

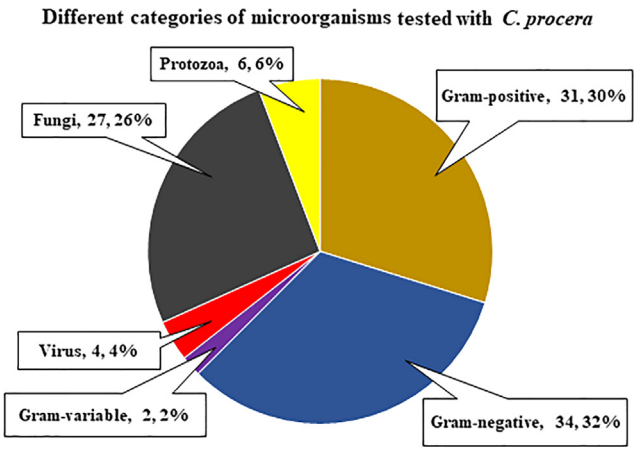

Fig. 1 Numbers and percentages of microorganisms that have been tested with *C. procera*.

AgNPs at dose of 20 µL/well produced ZOIs of 11.5, 13.8 and 16.8 mm, respectively against *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia* sp. and *Escherichia coli* (*E. coli*), while at dose of 50 µL/wells produced ZOIs of 23, 24, and 26 mm against *Aspergillus terreus, Trichophyton rubrum* (*T. rubrum*) AUMC 1804, and *Candida albicans* (*C. albicans*) AUMC 3880, respectively. However, the authors did not report the concentration of the AgNPs solution or suspension they used in the assay (Mohamed et al., 2014). In a different study, CPL was used as the capping agent in synthesising AgNPs and tested at doses of 2.5–12.5 µg/wells against selected bacteria (*E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538P and *B. subtilis* ATCC 6633), and fungi (*C. albicans* ATCC 10231, *A. niger* ATCC 16404). The findings showed varied degrees of *in vitro* antimicrobial effects. The AgNPs at the highest dose produced ZOI of 16.2 ± 0.5–21.2 ± 0.2, and 20.7 ± 2.1–22.2 ± 1.2 mm against the tested bacteria and fungi, respectively. The activity was significantly more potent than that of crude CPL and CPL extracts (Hassan et al., 2017). In another study, *C. procera* laticifer proteins (CPLP) at doses of 0.2 and 1% have been used in developing of a PVA-based bio-membrane as a delivery system that showed significant *in vivo* wound healing effect in mice model (De Figueiredo et al., 2014).

3. Isolated phytochemicals of *C. procera* and their antimicrobial activities

Thus far, only limited authors investigated antimicrobial properties of some isolated phytochemicals of *C. procera*, as discussed in the following sections:

3.1. C. procera cardenolide derivatives and their antimicrobial effects

Currently, 36 different cardenolides were reported for *C. procera* (Rajagopalan et al., 1955; Bruschweiler et al., 1969; Elgamal et al., 1999; Hanna et al., 1999, 2002; Mohamed et al., 2015; Sweidan & Zarga, 2015), out of which five aglycones, and seven cardenolide glycosides have been investigated for antimicrobial properties.

Proceragenin **(1)**, a cardenolide isolated from MeOH extract of *C. procera* was screened against a panel of 12 bacterial strains consisted of six G–ve (*Aeromonas caviae, Aeromonas sobriae, E. coli* (N-97–4), *K. pneumoniae* (U-671), *Pseudomonas pseudomalliae,* and *Vibrio cholerae* (N.C-58)) and six G + ve strains (*B. subtilis, Corynebacterium diphtheriae, Corynebacterium pseuedodiphthericum, Micrococcus luteus* (*M. luteus*), *Streptococcus agalactiae,* and *Streptococcus faecalis*). Compound **(1)** at dose of 150 µg/well produced ZOI values of 20–30 and 16–27 mm and MIC values of 90–150 and 100–150 µg/mL against the G–ve and G + ve strains, respectively. *Aeromonas sobriae* and *S. faecalis* showed the largest ZOI values (Akhtar et al., 1992). In another study, eleven CPL isolated cardenolides namely: afrogenin **(2)**, 12*β*-hydroxy carpogenin **(3)**, 12*β*-hydroxy coroglaucigenin **(4)**, 3-*Epi*,12*β*-hydroxycoroglaucigenin **(5)**, calactin **(6)**, 15*β*-hydroxy calactin **(7)**, 3′*β*-methoxy-15*β*-hydroxy calactin **(8)**, calotoxin **(9)**, afroside **(10)**, Uscharin **(11)**, and 15*β*-hydroxy uscharine **(12)** were screened for *in vitro* antimicrobial effects against *C. albicans* and four bacteria (*B. subtilis, Mycobacterium bovis* (*M. bovis*) BCG, *E. coli* and MRSA). All of the compounds showed MIC > 80 µg/mL against *M. bovis* and MIC > 100 µg/mL against other tested microorganisms (Mohamed et al., 2015). Molecular structures of *C. procera* isolated aglycones (genins) and their glycoside derivatives are shown in Fig. 2.

3.2. C. procera flavonoids and their antimicrobial effects

Four flavonoids e.g., quercetin-3-*O*-rutinosides or rutin **(13)**, kaempferol-3-*O*-rutinoside **(14)**, isorhamnetin-3-*O*-rutinoside **(15)**, and 5-hydroxy-3,7-dimethoxyflavone-4′-*O*-β-glucopyranoside **(16)** isolated from MeOH extract of *C. procera* leaves were tested against a group of both G + ve and G–ve bacteria and a panel of fungi (*C. albicans*, *C. tropicalis*, *S. cerevisiae*, *A. niger*, *A. flavus*, and *P. chrysogenum*). Rutin **(13)** was the most potent compound that produced ZOIs of 11.5–22.0 mm, and MICs of 80–640 µg/mL against tested bacteria, while *B. subtilis* and *S. aureus* were the most sensitive strains. Similarly, compound **(13)** produced ZOI of 12.0–22.5 mm and MICs of 80–320 µg/mL against tested fungi, while the yeast species were most sensitive (Nenaah, 2013a). However, the antimicrobial MOA of *C. procera* isolated flavonoids specifically against the tested microorganisms was not investigated by the authors. Molecular structures of *C. procera* isolated antimicrobial flavonoids are shown in Fig. 2.

Flavonoids are potent natural antioxidants, and recently attracted more attentions due to their multiparous biological activities including antimicrobial effects against bacteria, fungi and viruses. Studies showed that some flavonoids even exhibited inhibitory activity against some resistant microbial strains through reversing or antagonising the resistance mechanisms of pathogens (Sato et al., 2004; Chan et al., 2013; Gupta & Pandey, 2020). Moreover, flavonoids contribute in synergistic antimicrobial effects if combined with other antibacterial compounds and antibiotics. For example, two weak antimicrobial flavones (chrysosplenol-D and chrysoplenetin) while combined with sub-inhibitory dose of berberine, they showed a potent effect against *S. aureus* via inhibition of MDR-pump of the bacterium (Stermitz et al., 2002).

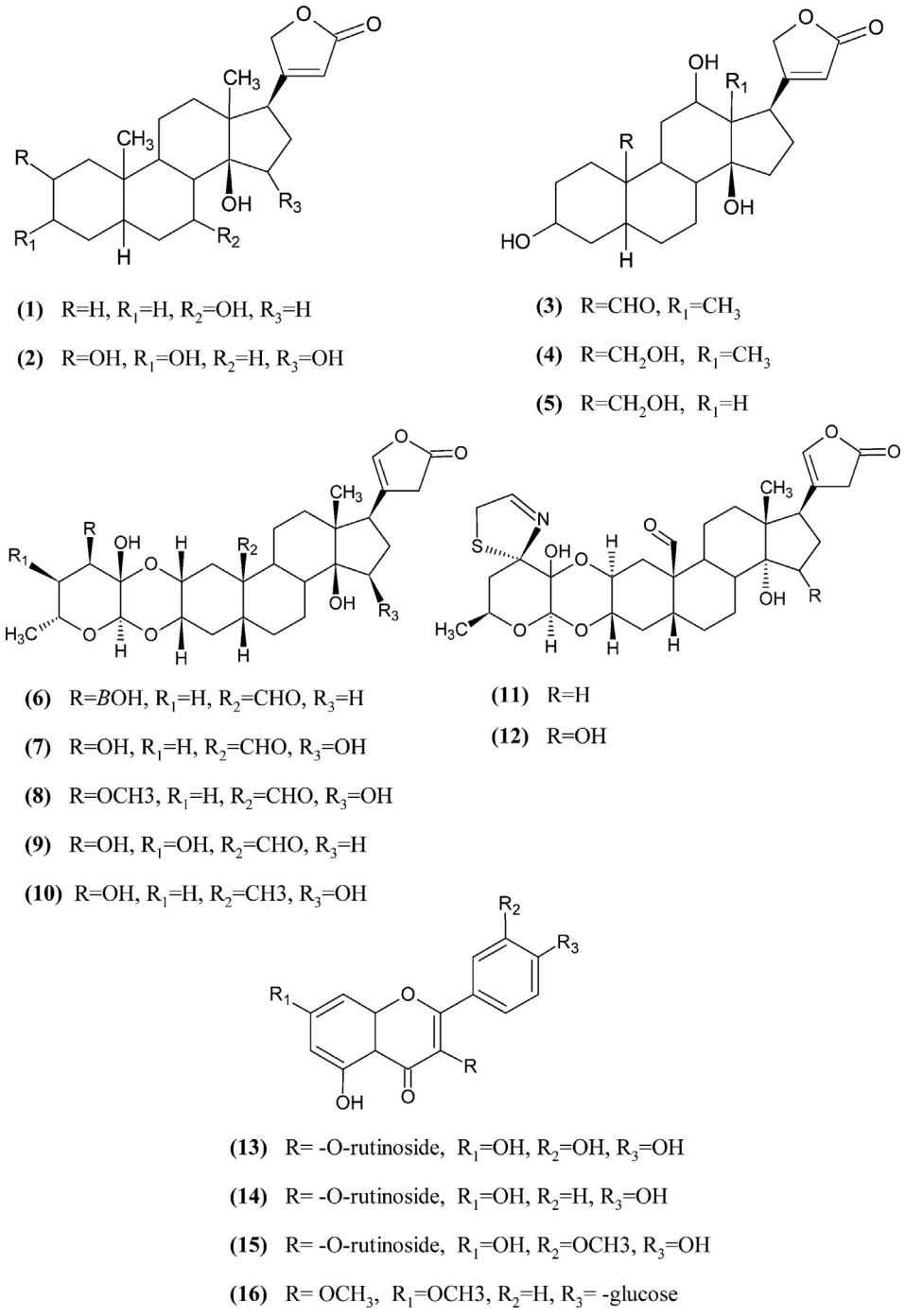

Fig. 2 Chemical structures of *C. procera* isolated compounds tested for *in vitro* antimicrobial activity.

Flavonoids can form complexes with the bacterial cell wall, extracellular components, soluble proteins, phosphate and glutamate from bacteria that eventually leads to disruption of cell wall peptidoglycan and attenuation of bacterial membrane permeability. Thus, the common antibacterial MOA of flavonoids could be via direct or indirect damaging of bacterial cellular membrane or cell wall (Wu et al., 2013; Gupta & Pandey, 2020). Flavonoids may also inhibit important enzymatic pathways of bacteria (Nenaah, 2013a), as their hydroxyl (—OH) groups contribute in their binding (through hydrogen bonds) with the enzymes' active sites and consequently alter their metabolism and lipid solubility. Overall, plants' flavonoids are considered as ideal antimicrobial compounds due to their lower toxicity (Wu et al., 2013; Gupta & Pandey, 2020) to overcome with the resistant infections.

3.3. C. procera isolated/purified proteins and peptides and their antimicrobial effects

C. procera is rich in proteins, peptides and different enzymes, some of which have been purified or isolated from CPL and different parts of the plant. Few purified proteins

and enzymes of *C. procera* were evaluated for their antimicrobial properties.

Osmotin (CpOsm) a polypeptide isolated from CPL was tested at the dose of 22 µg/mL against the phytopathogenic fungus *Fusarium solani*. The protein revealed its antifungal activity via membrane permeabilisation mechanism. Interestingly, CpOsm retained its antifungal potential in different pH ranges of 3.0 to 9.0, and was stable at up to 75 °C temperature (de Freitas et al., 2011a). Similarly, CpOsm at dose of 50 µg/wells inhibited growth of fungi *F. solani*, *Neurospora* sp. and *Colletotrichum gloeosporioides* with IC_{50} values of 67.0, 57.5 and 32.1 µg/mL, respectively (de Freitas et al., 2011b). The antifungal MOA of CpOsm was proposed to be mediated through two-steps, first, binding of CpOsm with the fungal spores' cell wall, and second, perturbation of the spores' cell membrane by CpOsm. Based on atomic force microscopy analysis, CpOsm caused wrinkling and up to 80% volume reduction of the treated fungal spores which was due to CpOsm-induced leakage in spores' membrane and loss of cytoplasmic contents of the treated spores (Ramos et al., 2015). Osmotin along with some other antibacterial peptides is found in latex of plants and is well known to be active against microorganisms invading plants (Ramos et al., 2019). However, molecular mechanism of CpOsm antifungal potential is still not clearly known.

Another protein (Calo-protein) purified from aqueous extract of *C. procera* stem barks showed broad *in vitro* antibacterial effects at doses of 6.25–100 µg/disk against a panel of bacteria (*E. coli, E. aerogenes, P. mirabilis, P. vulgaris, P. aeruginosa* and *S. aureus*) responsible for skin infections. At the highest dose (100 µg/disc) Calo-protein produced ZOI of 16.5–30 mm which was comparable with that of standard chloramphenicol (30 µg/disc, ZOI: 10–29 mm) against tested bacteria, while *S. aureus* was the most susceptible strain. Moreover, the antibacterial potential of Calo-protein against the tested bacteria which commonly cause skin infections, was supported by its significant *in vivo* wound-healing effect in a mouse model (Samy & Chow, 2012). Similarly, the enzyme chitinases isolated from CPL revealed antifungal effect against *Colletotrichum gloeosporioides* through degradation of the fungal cell wall, and probably alteration of fungal cell membrane permeability. Chitinases are capable of hydrolysing chitin of fungal cell walls and exert their antifungal effects (Freitas et al., 2016).

In a recent study, different cysteine peptidases (CpCP 1–3) purified from CPL were screened for their antifungal potential. The peptidases showed good antifungal potential (IC_{50} ≈50 µg/mL) against two phytopathogenic fungi (*F. oxysporum* and *Colletotrichum acutatum*). The authors further evaluated the antifungal mechanism of actions of peptidases on *F. oxysporum* spores, and confirmed that the antifungal effects were imposed through fungal cells' membrane permeabilisation, morphological changes alongside leakage of cellular content, and induction of reactive oxygen species (ROS) (Freitas et al., 2020).

Chitinases and other enzymes e.g., peptidases or peptide enzymes and cysteine proteases exist in plants, and they are called as antimicrobial peptides (AMPs). These peptides exhibit their antimicrobial effects by binding to the microbial membrane, altering membrane permeabilization that eventually leads to rupture of microbial cells (Salas et al., 2015). Chitinases can damage fungal cell walls through a chitinolytic activity, and cause growth inhibition and death of the microorganisms (Ramos et al., 2019).

4. Future perspective and trends in antimicrobial research of *C. procera*

Considering the rich phytochemistry and wide-ranging antimicrobial potential of *C. procera* against diverse microorganisms, the plant is highly expected to contribute in development of new alternative antimicrobial drugs. In this regard, further advanced antimicrobial investigations of this plant are highly emphasized. Nevertheless, in light of reviewed literatures and also considering the previous authors' suggestions, here we enumerated future research opportunities regarding *C. procera*, as discussed in the following sections:

4.1. Exploration of C. procera aimed at development of systemic antibacterial formulations

Phytochemicals are promising sources of new drugs including antibiotics. The MOA suggested for phytochemicals are; a direct antibacterial action, modifying or breaking the antibiotic resistance, reducing the MIC of another antibiotic, and modulating host defense through immunomodulatory effects (Khameneh et al., 2019).

In a study, crude MeOH extracts of *C. procera* leaves and flowers in combination with standard antibiotics, doripenem and imipenem, showed a synergistic effect against carbapenem-resistant *Acinetobacter baumannii*. Therefore, the authors recommended isolation of the active compounds of the crude extracts (Tiwari et al., 2016). Meanwhile, several authors have already suggested further investigations of *C. procera* in order to isolate potent antibacterial constituents. For instance, isolation and identification of active antimicrobial compounds from *C. procera* whole plant (Morsy et al., 2016), leaves (Jeya & Veerapapgu, 2017; Akin-Osanaiye & Okhomina, 2018; Alhazmi et al., 2018; Bilal et al., 2020), flowers (Ranjit et al., 2012; Tiwari et al., 2016), stem barks and roots (Asfere et al., 2018) were suggested by different authors in order to develop new and safe antibiotics. Nenaah, (2013a) endorsed *C. procera* flavonoids as antimicrobial biorationals, while Ullah et al., (2017) proposed isolation and purification of lead compounds from *C. procera* stems and leaves to be used specifically as anti-*M. tuberculosis*.

In addition to some cardenolides, flavonoids, proteins and peptide enzymes which were isolated earlier from *C. procera* as antimicrobial compounds, the plant contains many other compounds e.g., glycosides, triterpenoids, alkaloids, steroids, tannins, phenolic compounds, anthocyanins, saponins, resins, fatty acids, different enzymes, etc. (Khan & Malik, 1989; Gupta et al., 2003; Mendki et al., 2005; Chundattu et al., 2011; Mohamed et al., 2015; de Sousa et al., 2018; Freitas et al., 2020), which are also interesting to be systematically explored for their antibacterial effects and relevant MOA. Thus, we also suggest further in-depth studies of *C. procera* compounds in combination with other failed antibiotics to evaluate their efficiency as antibacterial agents or antibacterial resistance breakers (ARBs) against the resistant bacteria. Potential ARBs are highly valued nowadays in antibiotics drug discovery.

It is also worth noting that different endophytic fungi have been isolated from *C. procera* (Khan et al., 2007; Rani et al., 2019b), and some of them have shown potent *in vitro* antibacterial properties against tested bacteria (Table 2). *C. procera* endophytic fungi have been recommended to be further explored for their antimicrobial effects (Aharwal et al., 2014; Nascimento et al., 2015; Rani et al., 2017), and hence, this could be another opportunity in drug discovery of new antibiotics. Since *C. procera* is distributed in many territories, it provides a wide variety of endophytes that need to be explored, as well.

4.2. Exploration of C. procera aimed at development of wound-dressings and topical antibacterial formulations

Based on the wound-healing processes and the time required for wounds to heal, the wounds are broadly classified as acute and chronic wounds. However, due to the different types of wounds, and also advancement in medical technology, different products as wound healing aids or wound-dressings have been developed (Ghomi et al., 2019). Application of wound-healing dressings are indispensable in management of severe infected wounds and chronic wounds like diabetic wounds (Jaric et al., 2018). Although there are >2000 marketed products including different wound-dressings for treatment of wounds (Alves et al., 2019), some of them are reported to cause unwanted side effects. Thus, there is still a huge demand for developing efficient and safe wound-healing products particularly for treatment of chronic wounds such as diabetic wounds. Herbal products have been used in wound treatment since long back, and commonly herbal wound-healing dressings are more preferred since they are nontoxic and could be used for a long time (Alves et al., 2019; Ghomi et al., 2019).

C. procera has been traditionally used in treatment of different dermal infections, injuries, cuts, boils and wounds (Table 1), and has shown a wide range of antimicrobial effects (Samy & Chow, 2012; Mohamed et al., 2014), significant *in vivo* wound-healing effects in different animal models (Rasik et al., 1999; Aderounmua et al., 2013; De Figueiredo et al., 2014; Patil & Makwana, 2015), and *in vitro* and *in vivo* anti-inflammatory effects (Mascolo et al., 1988; Kumar et al., 2011; Ramos et al., 2020).

In a study, the Calo-protein purified from *C. procera* stem barks showed potent *in vitro* antibacterial effects comparable to that of chloramphenicol against some bacterial strains responsible for skin infections. Similarly, the proteins showed a significant *in vivo* wound healing activity comparable to standard fusidic acid in experimental animal model. Thus, the authors recommended Calo-protein for the development of antibacterial drugs against wound infectious bacteria (Samy & Chow, 2012). Similarly, a PVA-based bio-membrane integrated with 0.2 and 1% *C. procera* laticifer proteins was developed that revealed significant *in vivo* wound healing effect in mice model, and was safe as well (De Figueiredo et al., 2014). The studies have shown that different natural compounds e.g., glycosides, flavonoids, triterpenoids, steroids, phenolics, saponins, fatty acids, peptides, amino acids and proteases efficiently promote the wound-healing processes (Urs et al., 2017; Jaric et al., 2018; Alves et al., 2019), and *C. procera* is documented to be rich in all such compounds. Thus, *C. procera* could be a suitable candidate for developing of wound-healing pharmaceutical formulations such as: skin patches or wound dressings for topical applications.

4.3. Exploration of C. procera aimed at development of topical antifungal formulations

Around one-fourth of world's population is suffering from cutaneous fungal infections (Pai et al., 2018). The emergence of antifungal drug resistance is likewise a paramount public health concern worldwide (Friedman & Schwartz, 2019). Taking into account the limited number of antifungal drugs, one of the essential strategies in treatment of fungal infection is to overcome antifungal resistance. Understanding of the resistance mechanism is important in developing appropriate antifungal therapy. Meanwhile, combination therapy facilitates synergistic effects of antifungal drugs, enhance further the activity spectrum (Pai et al., 2018), and will contribute in breaking the resistance mechanisms of the fungal pathogens.

C. procera, which is being used traditionally in treatment of dermatophytic infections, ringworm, and tinea capitis (Table 1), showed *in vitro* antifungal potential against various pathogenic fungi (Table 2). Few authors reported anti-candidial and anti-dermatophytic properties of some *C. procera* isolated compounds, as well. Consequently, previous authors also suggested further isolation of *C. procera* antifungal and anti-dermatophytic compounds, elucidation of their action mechanisms (Verma et al., 2011, 2012; Hassan et al., 2006; Aliyu et al., 2015), and development of tineacide antifungal formulations from CPL (Kuta, 2008). Hence, *C. procera* could be counted as the best candidate for further extensive researches and bioassay-guided investigations in order to isolate its potent antifungal compounds aimed to develop topical antifungal formulation (s).

4.4. Exploration of C. procera aimed at development of anti-malarial formulations

Drug resistant malaria is still a public health burden and there is an urgent need for identification of new anti-malarial drugs in order to combat with the resistant plasmodium (Singh et al., 2015). *C. procera* has been traditionally used in treatment of malarial fever and pains by local people (Muthaura et al., 2015). Furthermore, *in vitro* antiplasmodial potential of *C. procera* whole plant extract against *P. berghei* (Misra et al., 1991), and *in vitro* schizontocidal effects of crude extracts of *C. procera* (different parts) and its fractions has been evaluated against both chloroquine-sensitive and chloroquine resistant strains of *P. falciparum* (Sharma & Sharma, 1999, 2000; Muthaura et al., 2015). Strong *ex vivo* antimalarial potential of *C. procera* leaves had also been reported (Singh et al., 2015). As isolation and identification of *C. procera* potent anti-malarial compounds was recommended earlier (Sharma & Sharma, 2000), recent studies also confirmed that *C. procera* could be a suitable candidate for further advanced studies in order to characterise the active anti-plasmodial constituents of the plant. Further *in silico* studies and derivatisation strategies of *C. procera* phytochemicals would also be considered as future research opportunities.

4.5. Development of natural herbicides, insecticides and phytofungicides from C. procera

C. procera is enlisted among the invasive weeds in some countries due to its fast growing and drought tolerance natures (Pompelli et al., 2019). Besides the fast-growing capability of *C. procera* in both wet and dry environments, its potent constituents such as cardenolides, flavonoids, alkaloids, different enzymes, etc. could act as allelochemicals and suppress growth of some other plants. Allelopathic properties of *C. procera* have been evaluated by several authors (Radwan et al., 2019). Strong allelopathic properties of *C. procera* shoot, root, and leaf extracts were recently reported and the plant was suggested for investigation of allelochemicals that would contribute as natural herbicides or insecticides (Radwan et al., 2019; Hussain et al., 2020).

C. procera laticifer proteins (CPLP) showed insecticidal effects against different crop pests. The activity was attributed to the presence of chitin-binding proteins (e.g., chitinases) and their chitinolytic activity that damage peritrophic membranes of insects (Ramos et al., 2007, 2019). In a study, significant insecticidal activity of CPL proteins, 80% MeOH extract of *C. procera* leaves and crude flavonoid fraction have been reported against adults of *Sitophilus oryzae* (L) and *Rhyzopertha dominica* (F), the two worst insects of stored grains e.g., rice. Similarly, *C. procera* isolated flavonoids namely kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside, 5-hydroxy-3,7-dimethoxyflavone-4′-O-β-glucopyranoside, and quercetin-3-O-rutinoside (rutin) were tested for insecticidal effects. In this case, rutin (quercetin-3-O-rutinoside) revealed potent effects against the two tested insects (Nenaah, 2013b).

Similarly, aqueous extract of CPL at dose of 1% produced significant inhibition of feeding and mortality against alfalfa weevil larvae (Al-Doghairi & El Hag, 2003), while PetE extract of *C. procera* leaves revealed significant antifeedant effect against the 4th instar larvae of cotton leafworm (Abdel-Rahman & Al-Mozini, 2007). In another study, EtOH extract of *C. procera* leaves significantly inhibited growth of the phytopathogenic fungi *Alternaria alternata* (*A. alternata*) (Srivastava & Singh, 2015), while n-hexane fraction of MeOH extract of *C. procera* leaves showed potent *in vitro* inhibitory effect against *Macrophomina phaseolina*, a phytopathogenic fungus responsible for charcoal rot diseases of crop plants (Waheed et al., 2016).

Consequently, further research is encouraged to develop natural & biodegradable herbicides, insecticides and phytofungicides from the extracts or phytochemicals of *C. procera* to control fungal infections and pests of crop plants.

In terms of environmental health and safety, natural phytofungicides and herbicides are more ecofriendly and biodegradable, and there is a huge demand for such products worldwide. It is worth noting, that ecofriendly approaches using safe and natural chemicals is sought in controlling pests of crop plants and fungal diseases of agricultural plants. In this regard, plants are considered as the unique arsenals of potent natural compounds, and some of which are believed to function against pests and various pathogens (Chauhan et al., 2017).

5. Summary and conclusion

In order to combat the resistant pathogens and nosocomial infections, there is a huge demand of developing new alternative efficient antibiotics. In this regard, natural products and MPs are still the unique resources of antimicrobial compounds. Natural compounds e.g., phytochemicals have shown to act as natural synergism, antibiotics' resistance breakers and resistance modifiers, and hence, their combination with other conventional antibiotics is counted as a promising approach for developing new and efficient antimicrobial drugs. As such, understanding the MOA and structure activity relationships of isolated antimicrobial phytochemicals would be helpful in their derivatisation and further use in developing alternative potent antimicrobial formulations.

C. procera, being rich in various bioactive constituents, showed multiparous biological activities. Although the plant is reported for antimicrobial effects by the different crude extracts and few isolated compounds against a wide range of microorganisms, further advanced and systemic bioassay-guided studies are encouraged to isolate antimicrobial compounds from the plant, to elucidate of antimicrobial MOA of its constituents, and to study synergistic properties of its phytochemicals in combination with other failed conventional antibiotics. In addition, considering the vast number of different phytochemicals reported from *C. procera*, *in silico* studies are also recommended in order to explore binding capabilities of *C. procera* phytochemicals with different target proteins and virulence factors of the resistant microorganisms.

Considering the reported potent *in vitro* antibacterial potential of crude extracts of *C. procera* endophytic fungi, this could be of novice research opportunities. *C. procera* endophytes still need to be explored as sources of potent antimicrobial compounds.

Lastly, toxicity (particularly ocular toxicity) of *C. procera* latex and its homemade remedies should not be underestimated. Therefore, necessary health education is required to inform local community healers as well as their patients to prevent splashing of CPL and other *C. procera* preparations into eyes, to avoid internal/oral use of non-standardized local herbal preparations of the plant, and to not collect *C. procera* grown in polluted areas/environments for medicinal consumption.

Overall, *C. procera* by virtue of its rich phytochemistry, provides many research opportunities for its isolated potent compounds in order to develop antimicrobial drugs not only for human infections but also for management of animals' infections and agricultural plants' microbial diseases. However, a bioassay-guided isolation, chemical characterisation and molecular mechanism studies of potent antibacterial compounds of Malaysian *C. procera* aerial parts (e.g., stems, leaves, and flowers) is currently in progress as part of our research project, at faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam, Malaysia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbasi, A.M., Khan, M.A., Ahmad, M., Zafar, M., Jahan, S., Sultana, S., 2010. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal

communities of North-West Frontier Province, Pakistan. J. Ethnopharmacol. 128 (2), 322–335. https://doi.org/10.1016/j.jep.2010.01.052.

Abdel-Mageed, W.M., Mohamed, N.H., Liu, M., El-Gamal, A.A., Basudan, O.A., Ismail, M.A., Quinn, R.J., Liu, X., Zhang, L., Shoreit, A.A.M., 2016. Lipoxygenase inhibitors from the latex of Calotropis procera. Arch. Pharm. Res. https://doi.org/10.1007/s12272-016-0725-9.

Abdel-Rahman, H.R., Al-Mozini, R.N., 2007. Antifeedant and toxic activity of some plant extracts against larvae of cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). Pak. J. Biol. Sci. 10 (24), 4467–4472.

Abubakar, I.B., Ukwuani-Kwaja, A.N., Garba, A.D., Singh, D., Malami, I., Salihu, T.S., Muhammad, A., Yahaya, Y., Sule, S.M., Ahmed, S.J., 2020. Ethnobotanical study of medicinal plants used for cancer treatment in Kebbi state, North-west Nigeria. Acta Ecologica Sinica 1–21. https://doi.org/10.1016/j.chnaes.2020.02.007.

Adamu, H.M., Abayeh, O.J., Agho, M.O., Abdullahi, A.L., Uba, A., Dukku, H.U., Wufem, B.M., 2005. An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. J. Ethnopharmacol. 99 (1), 1–4. https://doi.org/10.1016/j.jep.2004.12.025.

Aderounmua, A.O., Omonisib, A.E., Akingbasotec, J.A., Makanjuolad, M., Bejide, R.A., Orafidiya, L.O., Adelusola, K.A., 2013. Wound-healing and potential anti-keloidal properties of the latex of *Calotropis procera* (Aiton) Asclepiadaceae in rabbits. Afr. J. Tradit. Complement. Altern. Med. 10 (3), 574–579. https://doi.org/10.4314/ajtcam.v10i3.28.

Aharwal, R.P., Kumar, S., Sandhu, S.S., 2014. Isolation and antibacterial property of endophytic fungi isolated from indian medicinal plant *Calotropis procera* (Linn.) R. Br. World J. Pharm. Pharmac. Sci. 3 (5), 678–691.

Ahmad, N., Anwar, F., Hameed, S., Boyce, M.C., 2011. Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of akk [*Calotropis procera* (Ait.) Ait. F.)]. J. Med. Plant Res. 5 (19), 4879–4887.

Ahmed, N., Mahmood, A., Mahmood, A., Sadeghi, Z., Farman, M., 2015. Ethnopharmacological importance of medicinal flora from the district of Vehari, Punjab province, Pakistan. J. Ethnopharmacol. 168, 66–78. https://doi.org/10.1016/j.jep.2015.02.048.

Ahmed, O.M., Fahim, H.I., Boules, M.W., Ahmed, H.Y., 2016. Cardiac and testicular toxicity effects of the latex and ethanolic leaf extract of *Calotropis procera* on male albino rats in comparison to abamectin. SpringerPlus 5 (1), 1–21. https://doi.org/10.1186/s40064-016-3326-7.

Akhtar, N., Malik, A., Ali, S.N., Kazmi, S.U., 1992. Proceragenin, an antibacterial cardenolide from *Calotropis procera*. Phytochemistry 31 (8), 2821–2824.

Akin-Osanaiye, B.C., Okhomina, L., 2018. Phytochemical and antibacterial activity of leaf extracts of *Calotropis procera* on some selected bacteria. Direct Res. J. Biol. Biotechnol. 4 (2), 16–21 https://doi.org/10.26765/DRJBB.2018.6941.

Akindele, P., Fatunla, O., Ibrahim, K., Afolayan, C., 2017. Antibacterial and phytochemical screening of *Calotropis procera* leaf extracts against vancomycin and methicillin resistant bacteria isolated from wound samples in hospital patients. J. Compl. Alternat. Med. Res. 2 (1), 1–14. https://doi.org/10.9734/jocamr/2017/30975.

Al-Doghairi, M.A., El Hag, E., 2003. Effect of several biopesticides on alfalfa weevil larvae, *Hypera brunneipennis* (Boheman). Pak. J. Biol. Sci. 6 (8), 777–781.

Al-douri, N.A., 2000. A survey of medicinal plants and their traditional uses in Iraq. Pharm. Biol. 38 (1), 74–79.

Al-Fatimi, M., 2019. Ethnobotanical survey of medicinal plants in central Abyan governorate, Yemen. J. Ethnopharmacol. 241, 111973. https://doi.org/10.1016/j.jep.2019.111973.

Al-Ghanayem, A.A., Al Sobeai, S.M., Al Hussaini, M.S., Joseph, B., Saadabi, A.M., 2017. Antibacterial activity of certain Saudi Arabian medicinal plants used in folk medicine against different groups of bacteria. Nusantara Biosci. 9 (4), 392–395 https://doi.org/10.13057/nusbiosci/n090409.

Al-Mezaine, H.S., Al-Amry, M.A., Al-Assiri, A., Fadel, T.S., Tabbara, K.F., Al-Rajhi, A.A., 2008. Corneal endothelial cytotoxicity of the *Calotropis procera* (ushaar) plant. Cornea 27 (4), 504–506. https://doi.org/10.1097/ICO.0b013e3181611c34.

Al-Mezaine, H.S., Al-rajhi, A.A., Al-assiri, A., Wagoner, M.D., 2005. *Calotropis procera* (Ushaar) Keratitis. Am. J. Ophthalmol. 391 (1), 199–202.

Al-Qarawi, A.A., Mahmoud, O.M., Sobaih, M.A., Haroun, E.M., Adam, S.E.I., 2001. A preliminary study on the anthelmintic activity of *Calotropis procera* latex against *Haemonchus contortus* infection in Najdi sheep. Vet. Res. Commun. 25 (1), 61–70. https://doi.org/10.1023/A:1026762002947.

Al-Sulaibi, M.A.M., Thiemann, C., Thiemann, T., 2020. Chemical constituents and uses of *Calotropis procera* and *Calotropis gigantea* – a Review (Part I - The plants as material and energy resources). Open Chem. J. 7, 1–15. https://doi.org/10.2174/1874842202007010001.

Al-Taweel, A.M., Perveen, S., Fawzy, G.A., Rehman, A.U., Khan, A., Mehmood, R., Fadda, L.M., 2017. Evaluation of antiulcer and cytotoxic potential of the leaf, flower, and fruit extracts of *Calotropis procera* and isolation of a new lignan glycoside. Evidence-Based Compl. Alternat. Med. 2017, 1–10. https://doi.org/10.1155/2017/8086791.

Al-Zuhairi, A.H., Khalaf, J.M., Jabbar, A.N., 2020. Influence of aqueous extract of *Calotropis Procera* leaves in subchronic poisoning of goat. Plant Arch. 20 (Supl. 1), 1523–1527.

Alencar, N.M.N., Figueiredo, I.S.T., Vale, M.R., Bitencurt, F.S., Oliveira, J.S., Ribeiro, R.A., Ramos, M.V., 2004. Anti-inflammatory effect of the latex from *Calotropis procera* in three different experimental models: peritonitis, paw edema and hemorrhagic cystitis. Planta Med. 70 (12), 1144–1149. https://doi.org/10.1055/s-2004-835842.

Alhazmi, H.A., Sultana, S., Khan, A., Al-bratty, M., 2018. GC-MS analysis and antimicrobial activity of ethanolic extract of *Calotropis procera* (Ait.) R. Br. leaves. J. Chem. Pharmac. Res. 10 (1), 45–49.

Ali-Seyed, M., Ayesha, S., 2020. *Calotropis* - A multi-potential plant to humankind: Special focus on its wound healing efficacy. Biocatal. Agric. Biotechnol. 28, 101725. https://doi.org/10.1016/j.bcab.2020.101725.

Ali, A., Ansari, A., Qader, S.A.U., Mumtaz, M., Saied, S., Mahboob, T., 2014. Antibacterial potential of *Calotropis procera* (flower) extract against various pathogens. Pakistan J. Pharmac. Sci. 27 (5), 1565–1569.

Ali, N.A.A., Julich, W.-D., Kusnick, C., Lindequist, U., 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol. 74 (2), 173–179. https://doi.org/10.1016/S0378-8741(00)00364-0.

Aliyu, R.M., Abubakar, M.B., Kasarawa, A.B., Dabai, Y.U., Lawal, N., Bello, M.B., Fardami, A.Y., 2015. Efficacy and phytochemical analysis of aqueous extract of *Calotropis procera* against selected dermatophytes. J. Intercul. Ethnopharmacol. 4 (4), 314–317. https://doi.org/10.5455/jice.20151012012909.

Alves, A.Q., Da Silva, V.A., Goes, A.J.S., Silva, M.S., De Oliveira, G.G., Bastos, I.V.G.A., De Castro Neto, A.G., Alves, A.J., 2019. The fatty acid composition of vegetable oils and their potential use in wound care. Adv. Skin Wound Care 32 (8), 1–8. https://doi.org/10.1097/01.ASW.0000557832.86268.64.

Alzahrani, H.S., Mutwakil, M., Sabir, J., Saini, K.S., Alarif, W.M., Rizgallah, M.R., 2017. Anticancer and antibacterial activity of Calotropis procera leaf extract. J. Basic Appl. Sci. Res. 7 (12), 18–25.

Amini, M.H., Ahmady, A., Zhakfar, A.M., Sediqi, M.N., Babak, G., 2019. Preliminary phytochemical profile, in vitro antioxidant and sun protective activities of *Alhagi pseudalhagi* and *Elaeagnus angustifolia* L. J. Pharmac. Res. Int. 31 (4), 1–13. https://doi.org/10.9734/jpri/2019/v31i430308.

Arya, S., Kumar, V.L., 2004. Interleukin-1β inhibits paw oedema induced by local administration of latex of *Calotropis procera* extracts. Inflammopharmacology 12 (4), 391–398. https://doi.org/10.1163/1568560043696254.

Asfere, Y., Kebede, A., Muthuswamy, M., 2018. In-vitro antimicrobial activities and phytochemical screening of Calotropis procera (Ait.) and Vernonia amygdalina (Del.) extracts against some medically important pathogenic bacteria. Am. J. Biosci. Bioeng. 6 (6), 42–55 https://doi.org/10.11648/j.bio.20180606.11.

Azhar, M.F., Siddiqui, M.T., Ishaque, M., Tanveer, A., 2014. Study of ethnobotany and indigenous use of *Calotropis procera* (Ait.) in Cholistan Desert, Punjab, Pakistan. J. Agric. Res. 52 (1), 117–126.

Aziz, M.A., Khan, A.H., Adnan, M., Izatullah, I., 2017. Traditional uses of medicinal plants reported by the indigenous communities and local herbal practitioners of Bajaur Agency, Federally Administrated Tribal Areas, Pakistan. J. Ethnopharmacol. 198 (August 2016), 268–281. https://doi.org/10.1016/j.jep.2017.01.024.

Bagla, V.P., McGaw, L.J., Eloff, J.N., 2012. The antiviral activity of six South African plants traditionally used against infections in ethnoveterinary medicine. Vet. Microbiol. 155 (2–4), 198–206. https://doi.org/10.1016/j.vetmic.2011.09.015.

Bahadur, S., Khan, M., Shah, M., Shuaib, M., Ahmad, M., Zafar, M., Begum, N., Gul, S., Ashfaq, S., Mujahid, I., Hussain, F., 2020. Traditional usage of medicinal plants among the local communities of Peshawar valley, Pakistan. Acta Ecologica Sinica 40, 1–29. https://doi.org/10.1016/j.chnaes.2018.12.006.

Basak, S.K., Bhaumik, A., Mohanta, A., Singhal, P., 2009. Ocular toxicity by latex of *Calotropis procera* (Sodom apple). Indian J. Ophthalmol. 57 (3), 232–234. https://doi.org/10.4103/0301-4738.49402.

Basu, A., Nag Chaudhuri, A.K., 1991. Preliminary studies on the antiinflammatory and analgesic activities of *Calotropis procera* root extract. J. Ethnopharmacol. 31, 319–324. https://doi.org/10.1016/0378-8741(91)90017-8.

Belvedere, G., La Terra, F., Manenti, M., Lortal, S., Codjia, J.C., Doko, S., Licitra, G., 2010. Investigation on coagulant properties of Calotropis procera and stabilization of its proteolytic enzymes Presented at 2010 Annual Meetings of the American Dairy Science Association (ADSA), Poultry Science Association (PSA), Asociación Mexicana de Producción Animal (AMPA), Canadian Society of Animal Science (CSAS), A J. Dairy Sci. 93 (Supplément 1).

Bezerra, C.F., Mota, É.F., Silva, A.C.M., Tomé, A.R., Silva, M.Z.R., de Brito, D., Porfírio, C.T.M.N., Oliveira, A.C., Lima-Filho, J.V., Ramos, M.V., 2017. Latex proteins from *Calotropis procera*: Toxicity and immunological tolerance revisited. Chem. Biol. Interact. 274, 138–149. https://doi.org/10.1016/j.cbi.2017.07.007.

Bhaskar, V.H., Ajay, S.S., 2009. Antimicrobial activity of *Calotropis procera* seeds. Asian J. Chem. 21 (7), 5788–5790.

Bhatia, H., Sharma, Y.P., Manhas, R.K., Kumar, K., 2014. Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India. J. Ethnopharmacol. 151, 1005–1018. https://doi.org/10.1016/j.jep.2013.12.017.

Biharee, A., Sharma, A., Kumar, A., Jaitak, V., 2020. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia 146, 104720. https://doi.org/10.1016/j.fitote.2020.104720.

Bilal, H., Ali, I., Uddin, S., Said, A., Ur Rahman, M., Khan, A.M., Shah, A.B., Khan, A.A., Khan, I., 2020. Biological evaluation of antimicrobial activity of *Calotropis procera* against a range of bacteria. J. Pharmac. Phytochem. 9 (1), 31–35.

Breckle, S.W., Hedge, I.C., Rafiqpoor, M.D., 2013. Vascular Plants of Afghanistan: an augmented checklist. Scientica Bonnensis.

Bruschweiler, F., Stocklin, W., Stockel, K., Reichstein, T., 1969. 208. Die Glykoside von *Calotropis procera* R. Br. Helvetica Chimica Acta 52 (7), 2086–2106.

Butt, M.A., Ahmad, M., Fatima, A., Sultana, S., Zafar, M., Yaseen, G., Ashraf, M.A., Shinwari, Z.K., Kayani, S., 2015. Ethnomedicinal uses of plants for the treatment of snake and scorpion bite in Northern Pakistan. J. Ethnopharmacol. 168, 164–181. https://doi.org/10.1016/j.jep.2015.03.045.

Carruthers, I.B., Griffiths, D.J., Home, V., Williams, L.R., 1984. Hydrocarbons from *Calotropis procera* in northern Australia. Biomass 4 (4), 275–282. https://doi.org/10.1016/0144-4565(84)90040-4.

Chan, B.C.L., Ip, M., Gong, H., Lui, S.L., See, R.H., Jolivalt, C., Fung, K.P., Leung, P.C., Reiner, N.E., Lau, C.B.S., 2013. Synergistic effects of diosmetin with erythromycin against ABC transporter over-expressed methicillin-resistant *Staphylococcus aureus* (MRSA) RN4220/pUL5054 and inhibition of MRSA pyruvate kinase. Phytomedicine 20, 611–614. https://doi.org/10.1016/j.phymed.2013.02.007.

Chan, E.W.C., Sweidan, N.I., Wong, S.K., Chan, H.T., 2017. Cytotoxic cardenolides from *Calotropis* species: A short review. Rec. Nat. Prod. 11 (4), 334–344 https://doi.org/10.25135/rnp.2017.1701.002.

Chan, E.W.C., Wong, S.K., Chan, H.T., 2016. Apocynaceae species with antiproliferative and/or antiplasmodial properties: A review of ten genera. J. Integ. Med. 14 (4), 269–284.

Chandrawat, P., Sharma, R.A., 2016. The Genus *Calotropis*: An overview on bioactive principles and their bioefficacy. Res. J. Recent Sci. 5 (1), 61–70.

Chauhan, K.R., Le, T.C., Chintakunta, P.K., Lakshman, D.K., 2017. Phyto-fungicides: structure activity relationships of the thymol derivatives against Rhizoctonia solani. J. Agric. Chem. Environ. 06 (04), 175–185. https://doi.org/10.4236/jacen.2017.64012.

Chundattu, S.J., Agrawal, V.K., Ganesh, N., 2011. Phytochemical investigation of *Calotropis procera*. Arab. J. Chem. 9 (August), S230–S234. https://doi.org/10.1016/j.arabjc.2011.03.011.

Chusri, S., Villanueva, I., Voravuthikunchai, S.P., Davies, J., 2009. Enhancing antibiotic activity: A strategy to control *Acinetobacter* infections. J. Antimicrob. Chemother. 64 (6), 1203–1211. https://doi.org/10.1093/jac/dkp381.

Danton, O., Somboro, A., Fofana, B., Diallo, D., Sidibé, L., Rubat-Coudert, C., Marchand, F., Eschalier, A., Ducki, S., Chalard, P., 2019. Ethnopharmacological survey of plants used in the traditional treatment of pain conditions in Mali. J. Herbal Med. 17–18, 100271. https://doi.org/10.1016/j.hermed.2019.100271.

De Figueiredo, I.S.T., Ramos, M.V., Ricardo, N.M.P.S., Gonzaga, M.L.D.C., Pinheiro, R.S.P., De Alencar, N.M.N., 2014. Efficacy of a membrane composed of polyvinyl alcohol as a vehicle for releasing of wound healing proteins belonging to latex of *Calotropis procera*. Process Biochem. 49 (3), 512–519. https://doi.org/10.1016/j.procbio.2013.12.015.

de Freitas, C.D.T., Lopes, J.L., Beltramini, L., de Oliveira, R.S., Oliveira, J.T., Ramos, M.V., 2011a. Osmotin from *Calotropis procera* latex: New insights into structure and antifungal properties. Biochimica et Biophysica Acta - Biomembranes 1808 (10), 2501–2507. https://doi.org/10.1016/j.bbamem.2011.07.014.

de Freitas, C.D.T., Nogueira, F.C., Vasconcelos, I., Oliveira, J.T., Domont, G., Ramos, M., 2011b. Osmotin purified from the latex of *Calotropis procera*: Biochemical characterization, biological activity and role in plant defense. Plant Physiol. Biochem. 49 (7), 738–743. https://doi.org/10.1016/j.plaphy.2011.01.027.

de Sousa, L.V., Santos, A.P.B., Di Souza, L., Santos, A.G.D., Beatriz, A., 2018. Evaluation of the properties of *Calotropis procera* oil aiming the production of biodiesel. Orbital 10 (2), 147–152 https://doi.org/10.17807/orbital.v10i2.1061.

Desta, B., 1993. Ethiopian traditional herbal drugs. Part II: Antibacterial activity of 63 medicinal plants. J. Ethnopharmacol. 39, 129–139.

Diarra, N., Klooster, C.V., Togola, A., Diallo, D., Willcox, M., de Jong, J., 2015. Ethnobotanical study of plants used against malaria in Sélingué subdistrict, Mali. J. Ethnopharmacol. 166, 352–360. https://doi.org/10.1016/j.jep.2015.02.054.

Doshi, H., Satodiya, H., Thakur, M.C., Parabia, F., Khan, A., 2011. Phytochemical screening and biological activity of *Calotropis Procera* (Ait). R. Br. (Asclepiadaceae) against selected bacteria and *Anopheles stephansi* larvae. *International*. J. Plant. Res. 1 (1), 29–33. https://doi.org/10.5923/j.plant.20110101.05.

Dubey, D., Sahu, M.C., Rath, S., Paty, B.P., Debata, N.K., Padhy, R.N., 2012. Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria. Asian Pacific J. Tropical Biomed. 2 (2 SUPPL.), S846–S854. https://doi.org/10.1016/S2221-1691(12)60322-0.

El-kamali, H.H., 2009. Ethnopharmacology of medicinal plants used in North Kordofan (Western Sudan). Ethnobotanical Leaflets 13, 203–210.

El-seedi, H.R., Khalifa, S.A.M., Yosri, N., Khatib, A., Chen, L., Saeed, A., Efferth, T., Verpoorte, R., 2019. Plants mentioned in the islamic scriptures (Holy Qur' ân and Ahadith): Traditional uses and medicinal importance in contemporary times. J. Ethnopharmacol. 243, 112007. https://doi.org/10.1016/j.jep.2019.112007.

Elgamal, M.H.A., Hanna, A.G., Morsy, N.A.M., Duddeck, H., Simon, A., Gáti, T., Tóth, G., 1999. Complete 1H and 13C signal assignments of 5α-cardenolides isolated from *Calotropis procera* R. BR. J. Mole. Struct. 477, 201–208. https://doi.org/10.1016/S0022-2860(98)00615-2.

Erdman, M.D., Erdman, B.A., 1981. *Calotropis procera* as a source of plant hydrocarbons. Econ. Bot. 35, 467–472. https://doi.org/10.1007/BF02858597.

Fahim, H.I., Ahmed, O.M., Boules, M.W., Ahmed, H.Y., 2016. Nephrotoxic effects of Abamectin and *Calotropis procera* latex and leaf extract in male Albino Rats. Am. J. Med. Med. Sci. 6 (3), 73–86. https://doi.org/10.5923/j.ajmms.20160603.03.

Fatima, A., Ahmad, M., Zafar, M., Yaseen, G., Khan, M.P.Z., Butt, M.A., Sultana, S., 2018. Ethnopharmacological relevance of medicinal plants used for the treatment of oral diseases in Central Punjab-Pakistan. J. Herbal Med. 12, 88–110. https://doi.org/10.1016/j.hermed.2017.09.004.

Filgona, J., Dunah, C.S., Wakshama, P.S., 2005. An in vitro study of the antimicrobial activity of the root extract of *Calotropis procera* and *Moringa oleifera*. Ife J. Sci. 7 (1), 43–46.

Fleurentin, J., Pelt, J.M., 1982. Repertory of drugs and medicinal plants of Yemen. J. Ethnopharmacol. 6 (1), 85–108. https://doi.org/10.1016/0378-8741(82)90073-3.

Freitas, C.D.T., Silva, R.O., Ramos, M.V., Porfírio, C.T.M.N., Farias, D.F., Sousa, J.S., Oliveira, J.P.B., Souza, P.F.N., Dias, L. P., Grangeiro, T.B., 2020. Identification, characterization, and antifungal activity of cysteine peptidases from *Calotropis procera* latex. Phytochemistry 169, 112163. https://doi.org/10.1016/j.phytochem.2019.112163.

Freitas, C.D.T., Viana, C.A., Vasconcelos, I.M., Moreno, F.B.B., Lima-Filho, J.V., Oliveira, H.D., Moreira, R.A., Monteiro-Moreira, A.C.O., Ramos, M.V., 2016. First insights into the diversity and functional properties of chitinases of the latex of *Calotropis procera*. Plant Physiol. Biochem. 108, 361–371. https://doi.org/10.1016/j.plaphy.2016.07.028.

Friedman, D.Z.P., Schwartz, I.S., 2019. Emerging fungal infections: New patients, new patterns, and new pathogens. J. Fungi 5 (67), 1–19. https://doi.org/10.3390/jof5030067.

Gairola, S., Sharma, J., Bedi, Y.S., 2014. A cross-cultural analysis of Jammu, Kashmir and Ladakh (India) medicinal plant use. J. Ethnopharmacol. 155 (2), 925–986. https://doi.org/10.1016/j.jep.2014.06.029.

Gajare, S.M., Patil, M.V., Mahajan, R.T., 2012. Phytochemical screening and antimicrobial activity of ethanol extract of *Calotropis procera* root. Int. J. Res. Phytochem. Pharmacol. 2 (3), 143–146.

Gherbawy, Y.A., Gashgari, R.M., 2013. Molecular characterization of fungal endophytes from *Calotropis procera* plants in Taif region (Saudi Arabia) and their antifungal activities. Plant Biosystems 148 (6), 1085–1092. https://doi.org/10.1080/11263504.2013.819043.

Ghomi, E.R., Khalili, S., Khorasani, S.N., Neisiany, R.E., Ramakrishna, S., 2019. Wound dressings: Current advances and future directions. J. Appl. Polym. Sci. 136 (27), 1–12. https://doi.org/10.1002/app.47738.

Gracia, C.A., Rangel-buitrago, N., Castro-barros, J.D., 2019. Non-native plant species in the Atlantico Department Coastal Dune Systems, Caribbean of Colombia: A new management challenge. Mar. Pollut. Bull. 141, 603–610. https://doi.org/10.1016/j.marpolbul.2019.03.009.

Gupta, A., Gupta, A., Rai, R., Siddiqui, I.R., Singh, J., 2002. Triterpenoid glycoside from *Calotropis procera*. Indian J. Chem. – Sect. B Organ. Med. Chem. 41B, 884–886. https://doi.org/10.1002/chin.200227215.

Gupta, A., Pandey, A.K., 2020. Antibacterial lead compounds and their targets for drug development. In: Egbuna, C., Kumar, S., Ifemeje, C., Ezzat, S.M., Kaliyaperumal, S. (Eds.), Phytochemicals as Lead Compounds for New Drug Discovery. 1st ed. Elsevier Inc., pp. 275–292. https://doi.org/10.1016/B978-0-12-817890-4.00018-4.

Gupta, A., Singh, R., Purwar, C., Chauhan, D., Singh, J., 2003. Two pentacyclic triterpenes from the stem of *Calotropis procera*. Indian J. Chem. – Sect. B Organ. Med. Chem. 42 (8), 2030–2033.

Halu, B., Vidyasagar, G.M., 2012. A comparative study: differential antimycoses activity of crude leaf extracts of *Calotropis* spp. Int. J. Pharm. Pharm. Sci. 4 (3), 4–7.

Hanna, A.G., Elgamal, M.H.A., Morsy, N.A.M., Duddeck, H., Kovacs, J., Toth, G., 1999. Two cardenolides from *Calotropis procera*. Magn. Reson. Chem. 37 (10), 754–757.

Hanna, A.G., Shalaby, N.M.M., Morsy, N.A.M., Simon, A., Tóth, G., Malik, S., Duddeck, H., 2002. Structure of a calotropagenin-derived artifact from *Calotropis procera*. Magn. Reson. Chem. 40 (9), 599–602. https://doi.org/10.1002/mrc.1057.

Hassan, L.M., Galal, T.M., Farahat, E.A., El-midany, M.M., 2015. The biology of *Calotropis procera* (Aiton) W.T. Trees – Struct. Funct. 29 (2), 311–320. https://doi.org/10.1007/s00468-015-1158-7.

Hassan, M.H.A., Ismail, M.A., Moharram, A.M., Shoreit, A.A.M., 2017. Phytochemical and antimicrobial of latex serum of *Calotropis procera* and its silver nanoparticles against some reference pathogenic strains. J. Ecol. Health Environ. 5 (3), 65–75 https://doi.org/10.18576/jehe/050301.

Hassan, N., Din, M.U., Hassan, F.U., Abdullah, I., Zhu, Y., Jinlong, W., Nisar, M., Iqbal, I., Wadood, S.F., Iqbal, S.S., Shah, S.I., Naeem, I., Sarwar, A., Ihsan, M., Khan, H., Zeb, U., 2020. Identification and quantitative analyses of medicinal plants in Shahgram valley, district swat, Pakistan. Acta Ecologica Sinica 40, 44–51. https://doi.org/10.1016/j.chnaes.2019.05.002.

Hassan, S.W., Bilbis, F.L., Ladan, M.J., Umar, R.A., Dangoggo, S. M., Saidu, Y., Abubakar, M.K., Faruk, U.Z., 2006. Evaluation of antifungal activity and phytochemical analysis of leaves, roots, and stem barks extracts of *Calotropis procera* (Asclepiadaceae). Pak. J. Biol. Sci. 9 (14), 2624–2629.

Husain, S.Z., Malik, R.N., Javaid, M., Bibi, S., 2008. Ethonobotanical properties and uses of medicinal plants of Morgah Biodiversity Park, Rawalpindi. Pakistan J. Botany 40 (5), 1897–1911.

Hussain, F., Rasool, A., Aziz, K., Raisham, S., Aziz, S., Badshah, L., Hussain, W., 2020. Allelopathic inhibition of germination, seedling growth and cell division of selected plant species by *Calotropis procera* (Ait.) Ait. Plant Sci. Today 7 (1), 1–8 https://doi.org/10.14719/pst.2020.7.1.606.

Hussain, T., Arshad, M., Khan, S., Sattar, H., Qureshi, M.S., 2011. In vitro screening of methanol plant extracts for their antibacterial activity. Pak. J. Bot. 43 (1), 531–538. https://doi.org/10.1142/9789814354868_0024.

Hussain, W., Badshah, L., Ullah, M., Ali, M., Ali, A., Hussain, F., 2018. Quantitative study of medicinal plants used by the communities residing in Koh-e-Safaid Range, northern Pakistani-Afghan borders. J. Ethnobiol. Ethnomed. 14 (30), 1–18.

Idm'hand, E., Msanda, F., Cherifi, K., 2020. Ethnobotanical study and biodiversity of medicinal plants used in the Tarfaya Province. Morocco. Acta Ecol. Sinica 40 (2), 134–144. https://doi.org/10.1016/j.chnaes.2020.01.002.

Inngjerdingen, K., Nergård, C.S., Diallo, D., Mounkoro, P.P., Paulsen, B.S., 2004. An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. J. Ethnopharmacol. 92, 233–244. https://doi.org/10.1016/j.jep.2004.02.021.

Iqbal, Z., Lateef, M., Jabbar, A., Muhammad, G., Khan, M.N., 2005. Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. J. Ethnopharmacol. 102 (2), 256–261. https://doi.org/10.1016/j.jep.2005.06.022.

Iwalewa, E.O., Elujoba, A.A., Bankole, O.A., 2005. *In vitro* spasmolytic effect of aqueous extract of *Calotropis procera* on Guinea-pig trachea smooth muscle chain. Fitoterapia 76 (2), 250–253. https://doi.org/10.1016/j.fitote.2004.12.011.

Jain, S.C., Sharma, R., Jain, R., Sharma, R.A., 1996. Antimicrobial activity of *Calotropis procera*. Fitoterapia 67 (3), 275–277.

James, O.O., Rabiu, W.S.U., Ayinke, A.-B.A., Uzoma, N.K., Olufunsho, A., 2013. Preliminary anti-fungal activity of the aqueous bark extract of *Calotropis procera* (Asclepiadaceae). Nigerian Quarterly J. Hospital Med. 23 (4), 338–341.

Jamshidi-Kia, F., Lorigooini, Z., Amini-Khoei, H., 2018. Medicinal plants: Past history and future perspective. J. HerbMed Pharmacol. 7 (1), 1–7 https://doi.org/10.15171/jhp.2018.01.

Jaric, S., Kostic, O., Mataruga, Z., Pavlovic, D., Pavlovic, M., Mitrovic, M., Pavlovic, P., 2018. Traditional wound-healing plants used in the Balkan region (Southeast Europe). J. Ethnopharmacol. 211 (June 2017), 311–328. https://doi.org/10.1016/j.jep.2017.09.018.

Jato, D.M., Zainab, A., David, S., Okewole, P., Aliyu, A., Jacob, K. A., 2010. Toxicity assessment of the aqueous extract of *Calotropis procera* in rabbits. Toxicol. Lett. 196S (2010), S93–S94. https://doi.org/10.1016/j.toxlet.2010.03.339.

Jeya, K.R., Veerapapgu, M., 2017. Phytochemical screening and antibacterial activity of methanol leaf extract of *Calotropis procera* L. against clinical pathogens. *International Journal of Advanced.* Life Sci. 10 (1), 111–116.

Joshi, M., Kaur, S., 2013. In vitro evaluation of antimicrobial activity and phytochemical analysis of *Calotropis procera*, *Eichhornia crassipes* and *Datura innoxia* leaves. Asian J. Pharm. Clin. Res. 6 (SUPPL.5), 25–28.

Joshi, R.K., 2018. Role of natural products against microorganisms. Am. J. Clin. Microbiol. Antimicrob. 1 (1), 1–5.

Kar, D., Pattnaik, P.K., Pattnaik, B., Kuanar, A., 2018. Antimicrobial analysis of different parts extract in different solvent system of a waste weed- *Calotropis procera*. Asian J. Pharm. Clin. Res. 11 (2), 227–230 https://doi.org/10.22159/ajpcr.2018.v11i2.21081.

Karar, M., Kuhnert, N., 2017. Herbal drugs from Sudan: Traditional uses and phytoconstituents. Pharmacogn. Rev. 11, 83–103. https://doi.org/10.4103/phrev.phrev.

Kareem, S.O., Akpan, I., Ojo, O.P., 2008. Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms. Afric. J. Biomed. Res. 11 (1), 105–110. https://doi.org/10.4314/ajbr.v11i1.50674.

Kawo, A., Mustapha, A., Abdullahi, B., Rogo, L., Gaiya, Z., Kumurya, A., 2009. Phytochemical properties and antibacterial activities of the leaf and latex extracts of *Calotropis procera* (Ait.F.) Ait.F. *Bayero*. J. Pure Appl. Sci. 2 (1), 34–40. https://doi.org/10.4314/bajopas.v2i1.58453.

Khameneh, B., Iranshahy, M., Soheili, V., Bazzaz, B.S.F., 2019. Review on plant antimicrobials: A mechanistic viewpoint. Antimicrobial Resistance Infect. Control 8 (1), 1–28. https://doi.org/10.1186/s13756-019-0559-6.

Khan, A.Q., Ahmed, Z., Kazmi, S.N., Malik, A., 1988. A new pentacyclic triterpene from *Calotropis procera*. J. Nat. Prod. 51 (5), 925–928. https://doi.org/10.1021/np50059a018.

Khan, A.Q., Malik, A., 1989. A steroid from *Calotropis procera*. Phytochemistry 28 (10), 2859–2861.

Khan, R., Shahzad, S., Choudhary, M.I., Khan, S.A., Ahmad, A., 2007. Biodiversity of the endophytic fungi isolated from *Calotropis procera* (Ait.) R. Br. Pak. J. Bot. 39 (6), 2233–2239.

Khanzada, S.K., Shaikh, W., Kazi, T.G., Sofia, S., Kabir, A., Usmanghani, K., Kandhro, A.A., 2008. Analysis of fatty acid, elemental and total protein of *Calotropis procera* medicinal plant from Sindh, Pakistan. Pakistan J. Botany 40 (5), 1913–1921.

Khurana, S.M.P., Singh, S., 1972. Studies on *Calotropis procera* latex as inhibitor of Tobacco Mosaic Virus. J. Phytopathol. 73 (4), 341–346. https://doi.org/10.1111/j.1439-0434.1972.tb02556.x.

Kinda, P.T., Nacoulma, A.P., Guenné, S., Compaoré, M., Djandé, A., Lagnika, L., Kiendrébéogo, M., 2020. The metabolomic study of *Calotropis procera* Ait. from Burkina Faso, based on chemical functional groups profiling using FTIR. J. Compl. Integ. Med., 1–9 https://doi.org/10.1515/jcim-2019-0134.

Kipkore, W., Wanjohi, B., Rono, H., Kigen, G., 2014. A study of the medicinal plants used by the Marakwet Community in Kenya. J. Ethnobiol. Ethnomed. 10 (4), 1–22 https://doi.org/10.1186/1746-4269-10-24.

Konaté, K., Mavoungou, J.F., Lepengué, A.N., Aworet-Samseny, R. R.R., Hilou, A., Souza, A., Dicko, M.H., M'Batchi, B., 2012. Antibacterial activity against β- lactamase producing Methicillin and Ampicillin-resistants *Staphylococcus aureus*: Fractional Inhibitory Concentration Index (FICI) determination. Ann. Clin. Microbiol. Antimicrob. 11 (18), 1–12. https://doi.org/10.1186/1476-0711-11-18.

Kumar, S., Dewan, S., Sangraula, H., Kumar, V.L., 2001. Antidiarrhoeal activity of the latex of *Calotropis procera*. J. Ethnopharmacol. 76, 115–118. https://doi.org/10.1016/S0378-8741(01)00219-7.

Kumar, S., Goyal, S., Chauhan, A., Parveen, F., 2005. Some new ethnomedicinal uses of Milkweed in the Indian desert. Indian J. Tradit. Knowl. (IJTK) 04 (4), 448–455.

Kumar, V.L., Chaudhary, P., Ramos, M.V., Mohan, M., Matos, M. P.V., 2011. Protective effect of proteins derived from the latex of *Calotropis procera* against inflammatory hyperalgesia in monoarthritic rats. Phytother. Res. 25 (9), 1336–1341. https://doi.org/10.1002/ptr.3428.

Kumar, V.L., Guruprasad, B., Chaudhary, P., Fatmi, S.M.A., Oliveira, R.S.B., Ramos, M.V., 2015. Protective effect of proteins derived from *Calotropis procera* latex against acute inflammation in rat. Auton. Autacoid Pharmacol. 35 (1–2), 1–8. https://doi.org/10.1111/aap.12022.

Kuta, F.A., 2008. Antifungal effect of *Calotropis procera* stem bark on Epidermophyton flocosum and Trichophyton gypseum. Afr. J. Biotechnol. 7 (13), 2116–2118. https://doi.org/10.5897/AJB08.226.

Lakhtakia, S., Dwivedi, P.C., Choudhary, P., Chalisgaonkar, C., Rahud, J., 2010. Ocular toxicity of *Calotropis* - missing links. Indian J. Ophthalmol. 58 (2), 169. https://doi.org/10.4103/0301-4738.60074.

Larhsini, M., Oumoulid, L., Lazrek, H.B., Wataleb, S., Bousaid, M., Bekkouche, K., Jana, M., 2001. Antibacterial activity of some Moroccan medicinal plants. Phytother. Res. 15 (3), 250–252. https://doi.org/10.1002/ptr.815.

Lázaro, S.F., Fonseca, L.D., Fernandes, R.C., Tolentino, J.S., Martins, E.R., Duarte, E.R., 2012. Efeito do extrato aquoso do algodão de seda (*Calotropis procera* Aiton) sobre a eficiência reprodutiva do carrapato bovino. Revista Brasileira de Plantas Medicinais 14 (2), 302–305. https://doi.org/10.1590/S1516-05722012000200008.

Lee, Y.-S., Kang, O.-H., Choi, J.-G., Oh, Y.-C., Keum, J.-H., Kim, S.-B., Jeong, G.-S., Kim, Y.-C., Shin, D.-W., Kwon, D.-Y., 2010. Synergistic effect of emodin in combination with ampicillin or

oxacillin against methicillin-resistant *Staphylococcus aureus*. Pharm. Biol. 48 (11), 1285–1290. https://doi.org/10.3109/13880201003770150.

Lev-yadun, S., 1999. Articulated cork in *Calotropis procera* (Asclepiadaceae). Aliso: J. Syst. Evolut. Botany 18 (2), 161–163.

Lima-Filho, J.V., Patriota, J.M., Silva, A.F.B., Filho, N.T., Oliveira, R.S.B., Alencar, N.M.N., Ramos, M.V., 2010. Proteins from latex of *Calotropis procera* prevent septic shock due to lethal infection by *Salmonella enterica serovar Typhimurium*. J. Ethnopharmacol. 129 (3), 327–334. https://doi.org/10.1016/j.jep.2010.03.038.

Lottermoser, B.G., 2011. Colonisation of the rehabilitated Mary Kathleen uranium mine site (Australia) by *Calotropis procera*: Toxicity risk to grazing animals. J. Geochem. Explor. 111 (1–2), 39–46. https://doi.org/10.1016/j.gexplo.2011.07.005.

Madge, C., 1998. Therapeutic landscapes of the Jola, The Gambia. West Africa. *Health & Place* 4 (4), 293–311.

Mahmoud, O.M., Adam, S.E.I., Tartour, G., 1979a. The effects of *Calotropis procera* on small ruminants: I. Effects of feeding sheep with the plant. J. Comp. Pathol. 89, 241–250.

Mahmoud, O.M., Adam, S.E.I., Tartour, G., 1979b. The effects of *Calotropis procera* on small ruminants: II. Effects of administration of the latex to sheep and goats. J. Comp. Pathol. 89 (2), 251–263.

Mainasara, M.M., Aliero, B.L., Aliero, A.A., Dahiru, S.S., 2011. Phytochemical and antibacterial properties of *Calotropis procera* (Ait) R. Br. (Sodom Apple) fruit and bark extracts. Int. J. Modern Botany 1 (1), 8–11. https://doi.org/10.5923/j.ijmb.20110101.03.

Mainasara, M.M., Aliero, B.L., Aliero, A.A., Yakubu, M., 2012. Phytochemical and antibacterial properties of root and leaf extracts of *Calotropis procera*. Nigerian J. Basic Appl. Sci. 20 (1), 1–6 http://www.ajol.info/index.php/njbas/index.

Mako, G.A., Memon, A.H., Mughal, U.R., Pirzado, A.J., Bhatti, S. A., 2012. Antibacterial effects of leaves and root extract of *Calotropis procera* Linn. Pakistan J. Agric. Agric. Eng. Veterinary Sci. 28 (2), 141–149.

Mali, R.P., Rao, P.S., Jadhav, R.S., 2019. A review on pharmacological activities of *Calotropis procera*. J. Drug Deliv. Therapeutics 9 (3-s), 947–951 https://doi.org/10.22270/jddt.v9i3-s.2870.

Manduzai, A.K., Abbasi, A.M., Khan, S.M., Abdullah, A., Prakofjewa, J., Amini, M.H., Amjad, M.S., Cianfaglione, K., Fontefrancesco, M.F., Soukand, R., Pieroni, A., 2021. The importance of keeping alive sustainable foraging practices: Wild vegetables and herbs gathered by Afghan refugees living in Mansehra District, Pakistan. Sustainability 13 (3), 1–17. https://doi.org/10.3390/su13031500.

Mascolo, N., Sharma, R., Jain, S.C., Capasso, F., 1988. Ethnopharmacology of *Calotropis procera* flowers. J. Ethnopharmacol. 22, 211–221.

Mastanaiah, J., Prabhavathi, N.B., Srivani, T., 2012. *In vitro* anti bacterial activity of different solvent extracts of the plant *Calotropis procera*. Res. J. Pharm. Technol. 5 (8), 1066–1068.

Mendki, P.S., Salunke, B.K., Kotrar, H.M., Maheshwari, V.L., Mahulikr, P.P., Kothari, R.M., 2005. Antimicrobial and insecticidal activities of flavonoid glycosides from *Calotropis procera* L. for post-harvest preservation of pulses. Biopesticides Int. 1 (3,4), 193–200.

Meragiaw, M., Asfaw, Z., Argaw, M., 2016. The status of ethnobotanical knowledge of medicinal plants and the impacts of resettlement in Delanta, Northwestern Wello, Northern Ethiopia. Evidence-Based Compl. Alternative Med. 2016, 1–24. https://doi.org/10.1155/2016/5060247.

Misra, P., Pal, N.L., Guru, P.Y., Katiyar, J.C., Tandon, J.S., 1991. Antimalarial activity of traditional plants against erythrocytic stages of *Plasmodium berghei*. Pharm. Biol. 29 (1), 19–23. https://doi.org/10.3109/13880209109082843.

Mohamed, N.H., Ismail, M.A., Abdel-Mageed, W.M., Shoreit, A.A. M., 2014. Antimicrobial activity of latex silver nanoparticles using *Calotropis procera*. Asian Pacific J. Tropical Biomed. 4 (11), 876–883 https://doi.org/10.12980/APJTB.4.201414B216.

Mohamed, N.H., Liu, M., Abdel-Mageed, W.M., Alwahibi, L.H., Dai, H., Ismail, M.A., Badr, G., Quinn, R.J., Liu, X., Zhang, L., Shoreit, A.A.M., 2015. Cytotoxic cardenolides from the latex of *Calotropis procera*. Bioorg. Med. Chem. Lett. 25, 4615–4620. https://doi.org/10.1016/j.bmcl.2015.08.044.

Mohanraj, R., Rakshit, J., Nobre, M., 2010. Anti HIV-1 and antimicrobial activity of the leaf extracts of *Calotropis procera*. Int. J. Green Pharm. 4 (4), 242–246. https://doi.org/10.4103/0973-8258.74132.

Morsy, N., Al Sherif, E.A., Abdel-Rassol, T.M.A., 2016. Phytochemical analysis of *Calotropis procera* with antimicrobial activity investigation. Main Group Chem. 15 (3), 267–273. https://doi.org/10.3233/MGC-160206.

Mossa, J.S., Tariq, M., Mohsin, A., Ageel, A.M., Al-Yahya, M.A., Al-Said, M.S., Rafatullah, S., 1991. Pharmacological studies on aerial parts of *Calotropis procera*. Am. J. Chin. Med. 19 (3–4), 223–231.

Mudi, S.Y., Bukar, A., 2011. Anti-plasmodia activity of leaf extracts of *Calotropis procera* Linn. Biokemistri 23 (1), 29–34.

Mukhtar, M., Arshad, M., Ahmad, M., Pomerantz, R.J., Wigdahl, B., Parveen, Z., 2008. Antiviral potentials of medicinal plants. Virus Res. 131 (2), 111–120. https://doi.org/10.1016/j.virusres.2007.09.008.

Mulat, M., Khan, F., Muluneh, G., Pandita, A., 2020. Phytochemical profile and antimicrobial effects of different medicinal plant: Current knowledge and future perspectives. Curr. Tradit. Med. 6 (1), 24–42. https://doi.org/10.2174/2215083805666190730151118.

Muraina, I.A., Adaudi, A.O., Mamman, M., Kazeem, H.M., Picard, J., McGaw, L.J., Eloff, J.N., 2010. Antimycoplasmal activity of some plant species from northern Nigeria compared to the currently used therapeutic agent. Pharm. Biol. 48 (10), 1103–1107. https://doi.org/10.3109/13880200903505633.

Murti, Y., Yogi, B., Pathak, D., 2010. Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). Int. J. Ayurveda Res. 1 (1), 14–17. https://doi.org/10.4103/0974-7788.59938.

Muthaura, C.N., Keriko, J.M., Mutai, C., Yenesew, A., Gathirwa, J. W., Irungu, B.N., Nyangacha, R., Mungai, G.M., Derese, S., 2015. Antiplasmodial potential of traditional phytotherapy of some remedies used in treatment of malaria in Meru – Tharaka Nithi County of Kenya. J. Ethnopharmacol. 175, 315–323. https://doi.org/10.1016/j.jep.2015.09.017.

Mutwakil, M., Hajrah, N., Atef, A., Edris, S., Sabir, M., Al-ghamdi, A., Sabir, M., Nelson, C., Makki, R., Ali, H., El-domyati, F., Alhajar, A., Gloaguen, Y., Al-zahrani, H., Sabir, J., Jansen, R., Bahieldin, A., Hall, N., 2017. Transcriptomic and metabolic responses of *Calotropis procera* to salt and drought stress. BMC Plant Biol. 17 (231), 1–11. https://doi.org/10.1186/s12870-017-1155-7.

Nascimento, D.C.D.O., Ralph, M.T., Batista, J.E.C., Silva, D.M.F., Gomes-Filho, M.A., Alencar, N.M., Leal, N.C., Ramos, M.V., Lima-Filho, J.V., 2016. Latex protein extracts from *Calotropis procera* with immunomodulatory properties protect against experimental infections with *Listeria monocytogenes*. Phytomedicine 23 (7), 745–753. https://doi.org/10.1016/j.phymed.2016.03.012.

Nascimento, T.L., Oki, Y., Lima, D.M.M., Almeida-Cortez, J.S., Fernandes, G.W., Souza-Motta, C.M., 2015. Biodiversity of endophytic fungi in different leaf ages of *Calotropis procera* and their antimicrobial activity. Fungal Ecol. 14, 79–86. https://doi.org/10.1016/j.funeco.2014.10.004.

Naser, E.H., Kashmer, A.M., Abed, S.A., 2019. Antibacterial activity and phytochemical investigation of leaves of *Calotropis procera* plant in Iraq by GC-MS. Int. J. Pharmac. Sci. Res. 10 (4), 1988–1994 https://doi.org/10.13040/IJPSR.0975-8232.10(4).1988-94.

Nasr, A., 2020. Evaluation of the *in vitro* antileishmanial activities of bioactive guided fractionations of two medicinal plants. Trop. Biomed. 37 (1), 15–23.

Naz, A., Chowdhury, A., Chandra, R., Mishra, B.K., 2020. Potential human health hazard due to bioavailable heavy metal exposure via consumption of plants with ethnobotanical usage at the largest chromite mine of India. Environ. Geochem. Health 42 (12), 4213–4231. https://doi.org/10.1007/s10653-020-00603-5.

Neamsuvan, O., Tuwaemaengae, T., Bensulong, F., Asae, A., Mosamae, K., 2012. A survey of folk remedies for gastrointestinal tract diseases from Thailand's three southern border provinces. J. Ethnopharmacol. 144 (1), 11–21. https://doi.org/10.1016/j.jep.2012.07.043.

Nenaah, G., 2013a. Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. World J. Microbiol. Biotechnol. 29 (7), 1255–1262. https://doi.org/10.1007/s11274-013-1288-2.

Nenaah, G.E., 2013b. Potential of using flavonoids, latex and extracts from *Calotropis procera* (Ait.) as grain protectants against two coleopteran pests of stored rice. Ind. Crops Prod. 45, 327–334. https://doi.org/10.1016/j.indcrop.2012.12.043.

Nenaah, G.E., Ahmed, M.E., 2015. Antimicrobial activity of extracts and latex of *Calotropis procera* (Ait.) and synergistic effect with reference antimicrobials. Cancer Biol. 5 (3), 7–14.

Oladimeji, H.O., Nia, R., Essien, E.E., 2006. *In-vitro* anti-microbial and brine-shrimp lethality potential of the leaves and stem of *Calotropis procera* (Ait). Afric. J. Biomed. Res. 9 (3), 205–211. https://doi.org/10.4314/ajbr.v9i3.48906.

Oraibi, A.I., Hamad, M.N., 2018. Phytochemical investigation of flavanoid of *Calotropis procera* in Iraq, isolation and identification of rutin, quercitin and kampferol. J. Pharmac. Sci. Res. 10 (9), 2407–2411.

Pai, V., Ganavalli, A., Kikkeri, N.N., 2018. Antifungal resistance in dermatology. Indian J. Dermatol. 63 (5), 361–368. https://doi.org/10.4103/ijd.IJD_131_17.

Panchal, P., Singh, K., 2015. Antimicrobial activity of *Withania sominfera* and *Calotropis procera* on pathogenic strains. Int. J. Curr. Pharmac. Rev. Res. 7 (4), 76–78.

Panda, S.K., 2014. Ethno-medicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. J. Ethnopharmacol. 151 (1), 158–175. https://doi.org/10.1016/j.jep.2013.10.004.

Panda, S.K., Rout, S.D., Mishra, N., Panda, T., 2011. Phytotherapy and traditional knowledge of tribal communities of Mayurbhanj district, Orissa, India. J. Pharmac. Phytother. 3 (7), 101–113.

Parabia, F.M., Kothari, I.L., Parabia, M.H., 2008. Antibacterial activity of solvent fractions of crude water decoction of apical twigs and latex of *Calotropis procera* (Ait.) R. Br. Natl. Prod. Radiance 7 (1), 30–34.

Parihar, G., Balekar, N., 2016. *Calotropis procera*: A phytochemical and pharmacological review. Thai J. Pharm. Sci. 40 (3), 115–131.

Pathak, A.K., Argal, A., 2007. Analgesic activity of *Calotropis gigantea* flower. Fitoterapia 78 (1), 40–42. https://doi.org/10.1016/j.fitote.2006.09.023.

Pathania, S., Bansal, P., Gupta, P., Rawal, R.K., 2020. Genus Calotropis: A hub of medicinally active phytoconstituents. Curr. Tradit. Med. 6 (4), 312–331. https://doi.org/10.2174/2215083805666190619095933.

Patil, R.A., Makwana, A.B., 2015. Anti-hyperbilirubinemic and wound healing activity of aqueous extract of *Calotropis procera* leaves in Wistar rats. Indian J. Pharmacol. 47 (4), 398–402. https://doi.org/10.4103/0253-7613.161262.

Payal, C., Sharma, R.A., 2014. An overview on giant milkweed (*Calotropis procera* (Ait.). Ait. F.). J. Plant Sci. 3 (1), 19 https://doi.org/10.11648/j.jps.s.2015030101.13.

Pompelli, M.F., Mendes, K.R., Ramos, M.V., Santos, J.N.B., Youssef, D.T.A., Pereira, J.D., Endres, L., Jarma-Orozco, A., Solano-Gomes, R., Jarma-Arroyo, B., Silva, A.L.J., Santos, M.A., Antunes, W.C., 2019. Mesophyll thickness and sclerophylly among *Calotropis procera* morphotypes reveal water-saved adaptation to environments. J. Arid Land 11 (6), 795–810. https://doi.org/10.1007/s40333-019-0016-7.

Radwan, A.M., Alghamdi, H.A., Kenawy, S.K.M., 2019. Effect of *Calotropis procera* L. plant extract on seeds germination and the growth of microorganisms. Ann. Agric. Sci. 64 (2), 183–187. https://doi.org/10.1016/j.aoas.2019.12.001.

Rahmatullah, M., Kabir, A.A.B.T., Rahman, M.M., Hossan, M.S., Khatun, Z., Khatun, M.A., Jahan, R., 2010a. Ethnomedicinal practices among a minority group of Christians residing in Mirzapur village of Dinajpur district, Bangladesh. Adv. Natl. Appl. Sci. 4 (1), 45–51.

Rahmatullah, M., Sultan, S., Toma, T.T., Lucky, S., Chowdhury, M. H., Haque, W.M., Annay, M.E.A., Jahan, R., 2010b. Effect of *Cuscuta reflexa* stem and *Calotropis procera* leaf extracts on glucose tolerance in glucose-Induced hyperglycemic Rats and Mice. Afr. J. Tradit. Complement. Altern. Med. 7 (2), 109–112.

Rajagopalan, S., Tamm, C., Reichstein, T., 1955. 216. Die Glykoside der samen von *Calotropis procera* R.Br. Helv. Chim. Acta 38 (7), 1809–1824. https://doi.org/10.1002/hlca.19550380718.

Ramos, M.V., De Oliveira, R.S.B., Pereira, H.M., Moreno, F.B.M. B., Lobo, M.D.P., Rebelo, L.M., Brandão-Neto, J., De Sousa, J.S., Monteiro-Moreira, A.C.O., Freitas, C.D.T., Grangeiro, T.B., 2015. Crystal structure of an antifungal osmotin-like protein from *Calotropis procera* and its effects on *Fusarium solani* spores, as revealed by atomic force microscopy: Insights into the mechanism of action. Phytochemistry 119, 5–18. https://doi.org/10.1016/j.phytochem.2015.09.012.

Ramos, M.V., Demarco, D., da Costa Souza, I.C., de Freitas, C.D. T., 2019. Laticifers, latex, and their role in plant defense. Trends Plant Sci. 24 (6), 553–567. https://doi.org/10.1016/j.tplants.2019.03.006.

Ramos, M.V., Freitas, A.P.F., Leitão, R.F.C., Costa, D.V.S., Cerqueira, G.S., Martins, D.S., Martins, C.S., Alencar, N.M.N., Freitas, L.B.N., Brito, G.A.C., 2020. Anti-inflammatory latex proteins of the medicinal plant *Calotropis procera*: a promising alternative for oral mucositis treatment. Inflamm. Res. 69 (9), 951–966. https://doi.org/10.1007/s00011-020-01365-7.

Ramos, M.V., Freitas, C.D.T., Stanisçuaski, F., Macedo, L.L.P., Sales, M.P., Sousa, D.P., Carlini, C.R., 2007. Performance of distinct crop pests reared on diets enriched with latex proteins from *Calotropis procera*: Role of laticifer proteins in plant defense. Plant Sci. 173, 349–357. https://doi.org/10.1016/j.plantsci.2007.06.008.

Rani, R., Sharma, D., Chaturvedi, M., Yadav, J.P., 2017. Antibacterial activity of twenty different endophytic fungi isolated from *Calotropis procera* and time kill assay. Clin. Microbiol. 06 (03), 1–6. https://doi.org/10.4172/2327-5073.1000280.

Rani, R., Sharma, D., Chaturvedi, M., Yadav, J.P., 2019a. Phytochemical analysis, antibacterial and antioxidant activity of *Calotropis procera* and *Calotropis gigantea*. Natl. Prod. J. 9 (1), 47–60.

Rani, R., Sharma, D., Chaturvedi, M., Yadav, J.P., 2019b. Total phenolic content and *in vitro* antioxidant activity of endophytic fungi isolated from *Calotropis procerea* L. Curr. Bioact. Compd. 15 (2), 232–241.

Ranjit, P.M., Santhipriya, T., Nagasri, S., Chowdary, Y.A., Gopal, P.N.V., 2012. Preliminary phytochemical screening and antibacterial activities of ethanolic extract of *Calotropis procera* flowers against human pathogenic strains. Asian J. Pharm. Clin. Res. 5 (3), 127–131.

Rasik, A.M., Raghubir, R., Gupta, A., Shukla, A., Dubey, M.P., Srivastava, S., Jain, H.K., Kulshrestha, D.K., 1999. Healing potential of *Calotropis procera* on dermal wounds in Guinea pigs. J. Ethnopharmacol. 68, 261–266. https://doi.org/10.1016/S0378-8741(99)00118-X.

Rehman, K., Mashwani, Z.U.R., Khan, M.A., Ullah, Z., Chaudhary, H.J., 2015. An ethno botanical perspective of traditional medicinal plants from the Khattak tribe of Chonthra Karak, Pakistan. J. Ethnopharmacol. 165, 251–259. https://doi.org/10.1016/j.jep.2015.02.035.

Roy, S., Sehgal, R., Padhy, B.M., Kumar, V.L., 2005. Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. J. Ethnopharmacol. 102 (3), 470–473. https://doi.org/10.1016/j.jep.2005.06.026.

Sadat-Hosseini, M., Farajpour, M., Boroomand, N., Solaimani-Sardou, F., 2017. Ethnopharmacological studies of indigenous medicinal plants in the south of Kerman, Iran. J. Ethnopharmacol. 199, 194–204. https://doi.org/10.1016/j.jep.2017.02.006.

Saher, U., Javeed, A., Ashraf, M., Altaf, I., Ghafoor, A., 2018. Evaluation of antiviral and cytotoxic activity of *Calotropis procera* against foot and mouth disease virus. Int. J. Sci. Eng. Res. 9 (9), 236–253.

Salas, C.E., Badillo-Corona, J.A., Ramírez-Sotelo, G., Oliver-Salvador, C., 2015. Biologically active and antimicrobial peptides from plants. Biomed Res. Int. 2015. https://doi.org/10.1155/2015/102129.

Salem, H.A., Algalib, M.A.I., 2011. Quantities and locations of Usher plants in Sudan. J. Sci. Technol. 13 (3), 125–131.

Salem, W.M., Sayed, W.F., Haridy, M., Hassan, N.H., 2014. Antibacterial activity of *Calotropis procera* and *Ficus sycomorus* extracts on some pathogenic microorganisms. Afr. J. Biotechnol. 13 (32), 3271–3280. https://doi.org/10.5897/ajb2014.13981.

Samvatsar, S., Diwanji, V.B., 2000. Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. J. Ethnopharmacol. 73 (1–2), 313–316. https://doi.org/10.1016/S0378-8741(00)00274-9.

Samy, R.P., Chow, V.T.K., 2012. Pilot study with regard to the wound healing activity of protein from *Calotropis procera* (Ait.) R. Br. Evidence-Based Compl. Alternat. Med. 2012, 1–11. https://doi.org/10.1155/2012/294528.

Samy, R.P., Rajendran, P., Li, F., Anandi, N.M., Stiles, B.G., Ignacimuthu, S., Sethi, G., Chow, V.T.K., 2012. Identification of a novel *Calotropis procera* protein that can suppress tumor growth in breast cancer through the suppression of NF-κB pathway. PLoS ONE 7 (12), 1–7. https://doi.org/10.1371/journal.pone.0048514.

Sato, M., Tanaka, H., Oh-Uchi, T., Fukai, T., Etoh, H., Yamaguchi, R., 2004. Antibacterial activity of phytochemicals isolated from *Erythrina zeyheri* against Vancomycin-resistant *Enterococci* and their Combinations with Vancomycin. Phytother. Res. 18, 906–910. https://doi.org/10.1002/ptr.1556.

Sehgal, R., Arya, S., Kumar, V.L., 2005. Inhibitory effect of extracts of latex of *Calotropis procera* against *Candida albicans*: A preliminary study. Indian J. Pharmacol. 37 (5), 334–335.

Shah, A., Rahim, S., 2017. Ethnomedicinal uses of plants for the treatment of malaria in Soon Valley, Khushab, Pakistan. J. Ethnopharmacol. 200, 84–106. https://doi.org/10.1016/j.jep.2017.02.005.

Shamim, S., Ahmed, Fatima, L., 2019. Pharmacological actions and therapeutic uses of Aak (*Calotropis procera*): A review. Pharma Innovat. J. 8 (2), 40–47.

Sharma, A.K., Kharb, R., Kaur, R., 2011. Pharmacognostical aspects of *Calotropis procera* (Ait) R. Br. Int. J. Pharma Bio Sci. 2 (3), B-480–B-488.

Sharma, J., Gairola, S., Sharma, Y.P., Gaur, R.D., 2014. Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. J. Ethnopharmacol. 158, 140–206. https://doi.org/10.1016/j.jep.2014.10.004.

Sharma, P., Sharma, J.D., 1999. Evaluation of *in vitro* schizontocidal activity of plant parts of *Calotropis procera* - An ethnobotanical approach. J. Ethnopharmacol. 68 (1–3), 83–95. https://doi.org/10.1016/S0378-8741(99)00052-5.

Sharma, P., Sharma, J.D., 2000. *In-vitro* schizonticidal screening of *Calotropis procera*. Fitoterapia 71 (1), 77–79. https://doi.org/10.1016/S0367-326X(99)00121-5.

Shivkar, Y.M., Kumar, V.L., 2003. Anthelmintic activity of latex of *Calotropis procera*. Pharm. Biol. 41 (4), 263–265. https://doi.org/10.1076/phbi.41.4.263.15666.

Silva, M.C.C., da Silva, A.B., Teixeira, F.M., de Sousa, P.C.P., Rondon, R.M.M., Honrio, J.E.R., Sampaio, L.R.L., Oliveira, S.L., Holonda, A.N.M., de Vasconcelos, S.M.M., 2010. Therapeutic and biological activities of *Calotropis procera* (Ait.) R. Br. Asian Pacific J. Tropical Med. 3 (4), 332–336. https://doi.org/10.1016/S1995-7645(10)60081-8.

Simonsen, H.T., Nordskjold, J.B., Smitt, U.W., Nyman, U., Palpu, P., Joshi, P., Varughese, G., 2001. *In vitro* screening of Indian medicinal plants for antiplasmodial activity. J. Ethnopharmacol. 74 (2), 195–204. https://doi.org/10.1016/S0378-8741(00)00369-X.

Singh, N., Kaushik, N.K., Mohanakrishnan, D., Tiwari, S.K., Sahal, D., 2015. Antiplasmodial activity of medicinal plants from Chhotanagpur plateau, Jharkhand, India. J. Ethnopharmacol. 165, 152–162. https://doi.org/10.1016/j.jep.2015.02.038.

Srivastava, D., Singh, P., 2015. *In vitro* fungitoxic evaluation and GC-MS analysis of *Calotropis procera*. World J. Pharm. Res. 4 (3), 1123–1135.

Stermitz, F.R., Scriven, L.N., Tegos, G., Lewis, K., 2002. Two flavonols from Artemisa annua which potentiate the activity of berberine and norfloxacin against a resistant strain of *Staphylococcus aureus*. Planta Med. 68, 1140–1141.

Suleiman, M.H.A., 2015. An ethnobotanical survey of medicinal plants used by communities of Northern Kordofan region, Sudan. J. Ethnopharmacol. 176, 232–242. https://doi.org/10.1016/j.jep.2015.10.039.

Sweidan, N.I., Zarga, M.H.A., 2015. Two novel cardenolides from *Calotropis procera*. J. Asian Nat. Prod. Res. 17 (9), 900–907. https://doi.org/10.1080/10286020.2015.1040772.

Tanira, M.O.M., Bashir, A.K., Dib, R., Goodwin, C.S., Wasfi, I. A., Banna, N.R., 1994. Antimicrobial and phytochemical screening of medicinal plants of the United Arab Emirates. J. Ethnopharmacol. 41 (3), 201–205. https://doi.org/10.1016/0378-8741(94)90033-7.

Tapsoba, H., Deschamps, J.-P., 2006. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J. Ethnopharmacol. 104, 68–78. https://doi.org/10.1016/j.jep.2005.08.047.

Tariq, S., Wani, S., Rasool, W., Shafi, K., Bhat, M.A., Prabhakar, A., Shalla, A.H., Rather, M.A., 2019. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. Microb. Pathog. 134, 103580. https://doi.org/10.1016/j.micpath.2019.103580.

Tiwari, M., Roy, R., Tiwari, V., 2016. Screening of herbal-based bioactive extract against Carbapenem-resistant strain of *Acinetobacter baumannii*. Microbial Drug Resistance 00 (00), 1–8. https://doi.org/10.1089/mdr.2015.0270.

Tossou, M., Ballogou, B., Maina, J., Gicheha, M., 2018. Effect of *Calotropis procera* on the proximate composition and potential toxicity of Wagashi (traditional cheese) in Benin. Food Sci. Quality Manage. 74, 30–36.

Tounekti, T., Mahdhi, M., Khemira, H., 2019. Ethnobotanical study of indigenous medicinal plants of Jazan region, Saudi Arabia. Evidence-Based Compl. Alternat. Med. 1–45. https://doi.org/10.1155/2019/3190670.

Traore, M.S., Baldé, M.A., Diallo, M.S.T., Baldé, E.S., Diané, S., Camara, A., Diallo, A., Balde, A., Keita, A., Keita, S.M., Oulare, K., Magassouba, F., Diakite, I., Diallo, A., Pieters, L., Balde, A., 2013. Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. J. Ethnopharmacol. 150 (3), 1145–1153. https://doi.org/10.1016/j.jep.2013.10.048.

Ul-Zaman, H., Ahmad, S., 2017. Antibacterial activity and phytochemical analysis of leaf extracts of *Calotropis procera*. Acta Sci. Pharmac. Sci. 1 (5), 19–21.

Ullah, R., Hussain, Z., Iqbal, Z., Hussain, J., Khan, F.U., Khan, N., Muhammad, Z., Ayaz, S., Ahmad, S., Rehman, N.U., Hussain, I., 2010. Traditional uses of medicinal plants in Darra Adam Khel NWFP Pakistan. J. Med. Plants Res. 4 (17), 1815–1821. https://doi.org/10.5897/JMPR10.120.

Ullah, S., Hussain, S., Khan, S.N., Khurram, M., Khan, I., Khan, M. A., 2017. The medicinal plants in the control of Tuberculosis: Laboratory study on medicinal plants from the Northern Area of Pakistan. Int. J. Mycobacteriol. 6 (1), 102–105. https://doi.org/10.4103/ijmy.ijmy.

Urs, A.P., Manjuprasanna, V.N., Rudresha, G.V., Yariswamy, M., Vishwanath, B.S., 2017. Plant latex proteases: Natural wound healers. In: Chakraborti, S., Dhalla, N.S. (Eds.), Proteases in Physiology and Pathology. Springer Nature, pp. 297–323. https://doi.org/10.1007/978-981-10-2513-6_14.

Vadlapudi, V., Behara, M., Kaladhar, D.S.V.G.K., Suresh Kumar, S. V.N., Seshagiri, B., John Paul, M., 2012. Antimicrobial profile of crude extracts *Calotropis procera* and *Centella asiatica* against some important pathogens. Indian J. Sci. Technol. 5 (8), 3132–3136.

Velmurugan, S., Viji, V.T., Babu, M.M., Punitha, M.J., Citarasu, T., 2012. Antimicrobial effect of *Calotropis procera* active principles against aquatic microbial pathogens isolated from shrimp and fishes. Asian Pacific J. Tropical Biomed. 2 (2 SUPPL.), S812–S817. https://doi.org/10.1016/S2221-1691(12)60318-9.

Verma, R., Satsangi, G.P., Shrivastava, J.N., 2011. Susceptibility of a weed *Calotropis procera* (Ait.) against clinical isolates of dermatophytes. J. Med. Plant Res. 5 (19), 4731–4739 http://www.academicjournals.org/JMPR.

Verma, R., Satsangi, G.P., Shrivastava, J.N., 2012. Chemical analysis of leaves of weed *Calotropis procera* (Ait.) and its antifungal potential. Sect. A Health Perspect., 97–100 https://doi.org/10.1007/978-3-642-23394-4_20.

Vidyasagar, G.M., 2016. Plant-derived antifungal agents: Past and recent developments. In: Basak, A., Chakraborty, R., Mandal, S. M. (Eds.), Recent Trends in Antifungal Agents and Antifungal Therapy. Springer India, pp. 123–147. https://doi.org/10.1007/978-81-322-2782-3.

Waheed, N., Jabeen, K., Iqbal, S., Javaid, A., 2016. Biopesticidal activity of *Calotropis procera* L. against *Macrophomina phaseolina*. Afr. J. Tradit. Complement. Altern. Med. 13 (6), 163–167.

Waikar, S., Srivastava, V.K., 2015. *Calotropis* induced ocular toxicity. Medical Journal Armed Forces India 71 (1), 92–94. https://doi.org/10.1016/j.mjafi.2012.08.017.

WHO, 2019. 2019 Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline. World Health Organization. https://doi.org/World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO.

Wodah, D., Asase, A., 2012. Ethnopharmacological use of plants by Sisala traditional healers in northwest Ghana. Pharm. Biol. 50 (7), 807–815. https://doi.org/10.3109/13880209.2011.633920.

Wondimu, T., Asfaw, Z., Kelbessa, E., 2007. Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopia. J. Ethnopharmacol. 112, 152–161. https://doi.org/10.1016/j.jep.2007.02.014.

Wu, T., He, M., Zang, X., Zhou, Y., Qiu, T., Pan, S., Xu, X., 2013. A structure – activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. Biochimica et Biophysica Acta- Biomembranes 1828 (11), 2751–2756. https://doi.org/10.1016/j.bbamem.2013.07.029.

Yebouk, C., Redouan, F.Z., Benítez, G., Bouhbal, M., Kadiri, M., Ismail, A., Molero-mesa, J., Merzouki, A., 2020. Ethnobotanical study of medicinal plants in the Adrar Province, Mauritania. J. Ethnopharmacol. 246, 112217. https://doi.org/10.1016/j.jep.2019.112217.

Yesmin, M.N., Uddin, S.N., Mubassara, S., Akond, M.A., 2008. Antioxidant and antibacterial activities of *Calotropis procera* Linn. American-Eurasian J. Agric. Environ. Sci. 4 (5), 550–553.