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Green waste: A fresh approach to antimicrobial compounds

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ABSTRACT

One of the main objectives of (SDGs) is to prevent the environment and public health by substantially reducing waste generation through prevention, reduction, and recycling by 2030. Another issue of concern is the misuse and overuse of antimicrobials which are main drivers in the development of drug-resistant pathogens. The green waste was segregated and consisted of the flora of various families, extracted with three solvents, and the antibacterial activities through the disc diffusion method were observed on three most common pathogenic strains. Plant materials extracted with solvents manifest various degrees of antibacterial activities; ethyl acetate extracts draw the most promising results against the selected bacterial strains. The preliminary antimicrobial investigation concludes, given a scope of considerable research for green waste, the phytochemicals present can probably set off as one of the potential alternatives to the synthetic drugs and multidrug-resistant pathogens, and a way to reduce waste generation in the environment.

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1. Introduction

ne of the essential public services is waste collection and management to protect the environment and public health. The current global policy framework suggests that the waste services mainly target commitments to prevent, reduce, recycle, reuse, and adequately collect waste (urban solid waste) by 2030 (UNO, 2018). Green waste, mainly generated from the yards, is a section of urban solid waste that goes dumped unused. Biodegradable waste, which is carbon-based, is known as yard waste. The dry leaves, grass cuttings, twigs, paper, hay, sawdust, pines, needles, etc., come all under the yard waste (Tchobanoglous and Kreith, 2002). The brown waste and green waste (Fresh leaves; rich source of nitrogen) together is recycled by composting. Since the brown waste is needed only in a small amount for composting, the rest goes for dumping (Aruna et al., 2018). The present scenario in India is dealing with solid waste. Composting is one of the options to tackle the issue of handling solid waste (Sharma and Yadav, 2017). Despite recycling the waste as compost, a significant amount remains in the environment, further dumped in the dumpsites. The yard waste consists of leaves, grass, stems, non-woody plants, straw, etc., which contain a higher amount of lignocellulosic material, which draws a more extended time; the composting process can produce mulch in a short period. In sites where much yard waste is generated, especially during the rainy, spring, and autumn seasons, recycling these materials can be workable (Ghosh, 2016). Another issue of concern today is the misuse and overuse of antimicrobials, which are the main drivers in developing drug-resistant pathogens. Lack of clean water, sanitation, and inadequate infection prevention and control promote the spread of microbes, some of which can be resistant to antimicrobial treatment

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(WHO, 2017). The brown and the green waste together, called the yard waste, can be used for their secondary metabolites as antimicrobial agents, which are efficient for skin infections, food poisoning, intestinal infection, pneumonia, etc. (Alam and Ahmade, 2013). Since ancient times, the plant parts have been used as herbal medicines for their healing and medicinal properties. The plants consist of some bioactive compounds or secondary metabolites responsible for their medicinal properties. Some of the major bioactive compounds present in plants with medicinal values are tannins, alkaloids, phenolic compounds, flavonoids, etc. (Shihabudeen et al., 2010). Since the plant parts consist of phytochemicals, they blend different compounds. The composition may vary from plant to plant and affect the target organism as herbal medicine. Leaves and barks of medicinally important plants are known for antiinflammatory, anti-diabetic, anti-diarrhoeal, antifertility, and antifungal properties (Kalaria et al., 2012). They are also known for the treatment of gastrointestinal disorders, diarrhoea, toothaches, swelling, and cold (Temitope et al., 2013). Some medicinally important plant leaves have several properties like preventing hepatitis, controlling diabetes, antibacterial, etc. (Sarmiento et al., 2011). The traditional medicinal uses of several plants are known for the treatment of anxiety, eczema, fever, leprosy, skin inflammation, ulcers etc. (Nahar et al., 2021). One of the studies states the significant role of plant drug sources, which have been proven beneficial and effective in the cosmetic, fragrance as well as the food industries (Olaoluwa et al. 2022). More than 40 different species from several families have been reviewed for the ethnobotany, non-volatile compounds and biological activities to evaluate their potential as a natural product, for their application in different sectors (Mohammadhosseini et al. 2021a; Mohammadhosseini et al. 2021b; Mohammadhosseini et al. 2021c). In contrast, burns and bruises can be healed with the help of species of various plant families (Singh and Gupta, 2020). The reports available on the antimicrobial study of certain medicinally important species suggests that no such comparative reports of plant parts, especially the flower, has not been reported yet (Kar et al., 2018). Floral waste is always thrown without proper waste management. It can be reused or recycled for many value-added chemicals, bio-fertilizers, dyes and incense, biofuels, food supplements, etc. (Dutta et al., 2021). And besides this, it can also be used as an antimicrobial agent. The consideration of antimicrobial activities of various plants is of special engrossment nowadays because of the global issue of rising resistance to antibiotics for microorganisms. The continuous commercial use of these drugs has eventually led to the resistance of pathogenic microbes. The treatment of these microbes is crucial since they are dangerous to humans and can cause several infections if not treated. Development of novel medicines can be through phytochemicals as they blend many bioactive compounds and therefore are hard for the micro-organisms to get resistant to it. The present study was carried out for phytochemical extraction with three different solvent systems (acetone, ethyl acetate,

and petroleum ether) to make a comparative study against three virulent bacteria species (*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) to discover the antimicrobial properties of some common trees, shrubs and herbs found in India.

2. Experimental

2.1. Plant material

The primary plant families found in the green waste and can also be reused against multidrug-resistant strains of microbes are Apocynaceae, Meliaceae, Rutaceae, Asteraceae, Fabaceae, Malvaceae, etc. Therefore, fifteen different plant species were selected and further segregated as antimicrobials concerning the mentioned families. The tree species were chosen for the study consist of Alstonia scholaris, Azadirachta indica, Moringa olifera, Anona squamosa, Psidium quava, whereas the selected shrubs for the study consist of Lantana camera, Plumera rubra, Calotropis gigentia, Hibiscus rosa sinensis, Cascabela thevetia, the herbs include Ocimum sanctum, Cassia tora, Euphorbia hirta, Ipomea purpurea. Only the leaves were selected to study antimicrobials, whereas floral waste was chosen from a mixture of several flower parts collected from the waste. All the selected plants were then specified for qualitative analysis. Different tests were carried out to investigate the presence of alkaloids, flavonoids, terpenes, etc., which are accountable for antimicrobial activity (Shaikh and Patil, 2020). The green waste was collected from Nagpur, Maharashtra, India. Leaves were collected during the shedding and pruning of plants. In contrast, the floral waste was collected from religious places around Nagpur city, and the collected material was stored at room temperature in low humidity conditions.

2.2. Pre-processing of plant material

The plant materials were first segregated and then were finely chopped into small pieces and shade dried (green waste) thoroughly for 10-15 days, ground in the mechanical grinder to make a coarse powder. Each plant material coarse powder was stored in plastic bottles and labelled, respectively and stored at room temperature for further extraction. The selected plant parts were extracted with different solvents, such that all the polar and nonpolar compounds get drawn out in the crude extract.

2.3. Preparation of different solvent extracts

The selection of solvents and extraction methods were carried out with the help of a literature survey and setting up further evaluations, which concluded that maceration was the extraction method. Acetone, ethyl acetate, and petroleum ether were chosen as the extraction solvents. Each powdered plant material was extracted with acetone, petroleum ether, and ethyl acetate, respectively, by cold maceration. Powdered plant materials (100 g) were macerated with 300 mL of acetone, petroleum ether, and ethyl acetate separately



at room temperature for two days with frequent shaking. After two days, the extract was filtered using Whatman No. 1 filter paper. The filtrates (extracts) were evaporated to dryness using a rotary evaporator to yield the crude extracts and stored at 4 °C until further analysis. The extracts were labelled accordingly. The supernatant was filtered through Whatman filter paper while the residues were used for a second and third extraction. Percent extract yields of solvents and the mass of the extract in grams are given in Table 1 (Abubakar and Haque, 2020).

2.4. Test micro-organisms and growth media

Bacteria strains of *P. aeruginosa* (Gram-negative, ATCC 15442), *S. aureus* (Gram-positive, ATCC 6538), and *E. coli* (Gram-negative, ATCC 8739), were selected according to their pharmacological and clinical importance. The bacterial stock cultures were incubated for 24 h at 37 °C on nutrient agar (HiMedia), following refrigeration storage at 4 °C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37 °C (the bacteria were raised in the nutrient broth at 37 °C and maintained on nutrient agar slants at 4 °C). Antimicrobial susceptibility test (Disc diffusion method) was performed for all microbial isolates by modified Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guideline (Walker, 1999).

2.5. Antibacterial susceptibility test

Sterile filter disc (diameter 4 mm, Whatman paper No. 3) was placed in Petri dishes (diameter 90 mm) filled with MHA and seeded with 0.3 mL of the test organism. The disc was impregnated with test concentrations (12.5, 30, 50, and 100 μ g/mL) of the compounds investigated dissolved in concentrated (99.95%) dimethyl sulfoxide (DMSO). The growth inhibition zones around the discs were measured after 24 h of incubation at 37 °C. Each micro-organism was tested in triplicate, and the solvent (DMSO) was used as a control, while gentamycin (30 mcg) was used as a positive control (Bauer et al., 1966).

3. Results and Discussion

3.1. Yields of the plant extract

The green waste is generated mainly during the shedding and pruning of the plants. In contrast, floral waste is generated chiefly from religious places, and a large quantity is dumped without recycling. The selected plant parts were first dried, ground into a coarse powder, and extracted with different solvents, such that all the polar and non-polar compounds get drawn out in the crude extract. The selection of solvents and extraction methods were carried out with the help of a literature survey and setting up different evaluations, which concluded that maceration was the extraction method. Acetone, ethyl acetate, and petroleum ether were chosen as the extraction solvents. Additional tests were carried out to investigate the

presence of alkaloids, flavonoids, terpenes, etc., which are accountable for antimicrobial activity. Dragendroff's test, Hager's test, and Mayer's test were carried out to determine alkaloids in the extracts. In contrast, tests to assess flavonoids were carried out by the alkaline reagent test, ferric chloride test, and Conc. H₂SO₄ test. Phenolic compounds were determined with the help of an lodine test, ferric chloride test, and potassium dichromate test. Braymer's test and 10% NaOH test was carried out to determine the presence or absence of tannins, whereas terpenes were determined with Salkowski's test, and foam test, NaHCO, test was carried out to determine the presence or absence of saponins in the plant extracts and is depicted in Table 1 (Shaikh and Patil, 2020). The powdered plant materials were extracted by using the cold maceration method. The extract yield then went through an evaporation process using a rotary evaporator under 80 to 100 r/min at 60 °C to evaporate the surplus solvent. The crude extract was stored in an oven at 37 °C for the further drying process. The highest yield was obtained from A.scholaris in ethyl acetate extract (3.19%), while the minuscule yield was of Cascabela thevetia in ethyl acetate extract ((0.05%), Table 2).

3.2. Estimation of antimicrobial activity

The multidrug-resistant pathogens consist of several strains which do not respond to the synthetic drug treatment and have developed resistance to them and the changing environment (WHO, 2017). It is alarming since pathogens grow and spread quickly if not kept in a control state. The synthetic drugs used over time consisting of only one active ingredient are one of the primary reasons for developing resistance by the pathogens. The application of green drugs against such multidrug-resistant pathogens can be of great importance for inhibiting their multiplication and resistance. Likewise, the green waste generated can also be reused and recycled in an eco-friendly way, thus sustainably targeting the global policies. The crude extracts were first dissolved in concentrated (99.95%) DMSO so that it could get absorbed by the discs, and therefore, one control of DMSO (dimethyl sulfoxide) same concentration was added as the control to ensure it was identical to the test solutions which may have also contained the solvent and not affected the antibacterial activity. Another control of gentamycin (30 mcg) was kept to compare the effectiveness of antibacterial activity of the natural and synthetic products. These organisms were frequently noticed in infectious diseases. The study showed that all plant extracts used in the study exhibited a varying degree of antimicrobial activity against all micro-organisms tested. Initially, the micro-organisms were manifested in an extensive range of test concentrations to obtain the activity range of the extract solutions. Five concentrations ranging from 12.5-100 µg/mL yielding a maximum zone of inhibition in 24 h of exposure were selected for antibacterial bioassays. Total of three replicates were set, respectively for every concentration in which extracts



were dissolved and kept with each set of experiments. A zone of inhibition was recorded after 24 h (Fig. 1). The surrounding area around the disc with no growth of microbes to a visible distance from the radius of the disc was considered to have efficient antibacterial activity. The current study is carried out by selecting different plants characterized as yard waste on three microbial species at different concentrations to scrutinize the effective plants and suitable solvent systems as effective antibacterial agents; the following are the results discussed in the studies carried out.

The acetone extracts of *Alstonia scholaris*, Floral waste, *Azadirachta indica, Plumera rubra*, and *Euphorbia hirta* illustrated a significant antimicrobial activity for *S. aureus* by depicting zone of inhibition of 19.66 mm, 21.03 mm, 20.96 mm, 20.78 mm, 20.5 mm (100 µg/ mL), respectively. For *P. aeruginosa* the acetone extracts of *Alstonia scholaris*, Floral waste, *Azadirachta indica*, *Moringa oleifera*, and *Euphorbia hirta* depicted the zone of inhibition of 29 mm, 28 mm, 27 mm, 25 mm, 20 mm (100 µg/mL), respectively. Whereas for *E. coli* the acetone extracts of *Alstonia scholaris*, Floral waste, *Azadirachta indica, Plumera rubra, Ocimum sanctum*, and *Euphorbia hirta* delineated significant activity with the zone of inhibition of 22 mm, 17 mm, 20 mm, 19 mm, 19 mm, 20 mm (100 µg/mL), respectively (Fig. 2).

The ethyl acetate extracts of Alstonia scholaris, Floral waste, Azadirachta indica, Plumera rubra, Lantana camera, and Euphorbia hirta illustrated a significant antimicrobial activity for *S. aureus* by depicting the zone of inhibition of 21.8 mm, 23.9 mm, 23.8 mm, 19.4 mm, 18.2 mm, 18.2 mm (100 μ g/mL), respectively. For *P. aeruginosa* the ethyl acetate extracts of Alstonia scholaris, Floral waste, Azadirachta indica, and Ocimum sanctum depicted the zone of inhibition of 28.86 mm, 29 mm, 26.03 mm, 18 mm (100 μ g/mL), respectively. Whereas for *E. coli*, the ethyl acetate extracts of Alstonia scholaris, Floral waste, Azadirachta indica, and Euphorbia hirta delineated significant activity with the zone of inhibition of 20.33 mm, 19.36 mm, 20.03 mm, 18.1 mm (100 μ g/mL), respectively (Fig. 3).

The petroleum ether extracts of Alstonia scholaris, Floral waste, Azadirachta indica, Moringa oleifera, and Euphorbia hirta illustrated a significant antimicrobial activity for *S. aureus* by depicting the zone of inhibition of 21 mm, 23 mm, 15.93 mm, 20.6 mm, 23.5 mm (100 µg/mL), respectively. For *P. aeruginosa* the petroleum ether extracts of Alstonia scholaris, Floral waste, Azadirachta indica, Moringa oleifera, and Ipomoea purpurea depicted the zone of inhibition of 28.03 mm, 25.03 mm, 22.93 mm, 20.6 mm 18.1 mm (100 µg/mL), respectively. Whereas for E. coli, the petroleum ether extracts of Alstonia scholaris, Floral, Azadirachta indica, Plumera rubra, Annona squamosa, and Euphorbia hirta delineated significant activity with the zone of inhibition of 18.73 mm, 17.23 mm, 19.5 mm, 17.03 mm, 20.6 mm, 18.2 mm (100 µg/mL), respectively (Fig. 4).

3.3. Defence mechanism of phytochemicals

Plant families like Cladophoraceae, Miliaceae,

Asteraceae, Oocystaceae, Solanaceae, and Rutaceae consist of secondary metabolites that have various types of microbial and larval, adulticidal, and repellent activities against different species of mosquitoes (Shaalan et al., 2005). Many plants have described different groups of chemicals like terpenoids, alkaloids, essential oils, steroids, phenolic compounds, etc., as secondary metabolites used as a plant defence mechanism against various species of microbes and different invertebrates. Compounds like tannins are rich in protein content and can inhibit the regulation of protein synthesis by binding to proline-rich proteins (Shimada, 2006). The plant parts used in this study are rich in flavonoids, alkaloids, terpenes, saponins, tannins, and phenolic compounds, which the plants use as a defense mechanism against various microbial infections and active against multiple groups of microbes (Cowan, 1999). Further, it was observed that saponins could create leakage to specific proteins and enzymes from the cells, eventually resulting in the breakdown of the cells (Zablotowicz et al., 1996). One of the studies also depicts the production of flowers and the generation of floral waste globally. In these studies, the floral waste generated consists of potential secondary metabolites and can also generate value-added chemicals present in the floral waste, which is of significant importance and can be applied to various sectors, are critically reviewed. The value-added chemicals produced from the floral waste can be used as a substrate or co-substrate by application in pharmaceuticals, Biofertilizers, Environmental remediation, Biocidal, Bioenergy application, dyes, and soaps, incense sticks, nanoparticles, etc. One of the studies reviewed states that using flower extracts of different plants possesses potential secondary metabolites such as nematocides and pesticides (Dutta et al., 2021). Thiophene present in the Tagetes sp. Possess insecticidal properties and can be a potential candidate for pest control (Marotti et al., 2010). The synthesis of cadmium nanoparticles from the petals of Rosa sp. and Tagetes sp. Depicted 98.8% and 100% mortality against Aedes albopictus larvae, respectively (Hajra et al., 2016). The phytochemicals extracted from Magnolia kobus flowers illustrated, significant larvicidal, Adulticidal, and fumigant activities against Aedes albopictus (Kim et al., 2020). Plants comprise secondary metabolites which can act as natural agents to treat micro-organisms. The efficacy of biological antimicrobial agents has been more effective than the chemical antibiotic, to which the microorganisms have now become resistant. Brown and green waste are generally used for composting, but they can also be used as antimicrobial agents. The commonly available plants should be targeted for these activities. Such plants should be toxic to the micro-organisms causing various diseases. According to previous reports and the antibacterial studies carried out at present of different solvent extracts of Alstonia scholaris, Psidium guava, Azadirachta indica, Tagetes erecta, Plumera rubra, Moringa oliefera, Euphorbia hirta, Ocimum sanctum, etc. demonstrated antimicrobial activity against different micro-organisms. All the plant materials extracted with



Table 1

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Solvent	Qualitative phytochemical analysis	Alstonia scholaris	Floral waste	Azadirachta indica	Moringa oliefera	Plumera rubra	Lantana camera	Calotropis gigantia	Anona squamosa	Cassia tora	Hibiscus rosa sinensis	Psidium guava	Cascabela thevetia	Ocimum sanctum	Euphorbia hirta	Ipomea purpurea
	Alkaloids		÷	+			÷	+	+	÷	+		÷	+	+	+
	Flavanoids	+	+	+							+	+	+	+	+	+
Acetone	Terpenes	+		+			+	+	+	+		+	+			+
	Saponins	+	+	+	+	÷			+	+		+	+	+	+	
	Tannins	+	+	+	+	÷	+		+	+		+	+	+	+	+
	Phenolic compounds	+		+			+							+		+
	Alkaloids		+	+			÷			+	+		+	+	+	+
	Flavanoids	+	÷	+		÷					+	+	+	÷	+	+
Ethyl acetate	Terpenes	÷		+	÷		÷	+	÷	÷			÷			+
	Saponins	+	+	+	+	÷			+	+		+	+	+	+	
	Tannins	+	+	+	+	÷	+		+	+		+	+	+	+	+
	Phenolic compounds	+	+	-			+							+		+
	Alkaloids		+	-			+			+	+		+	+	+	+
	Flavanoids	-	÷	,		÷		-	-	,	+	÷	-	÷	÷	+
Petroleum ether	Terpenes		+		÷		+	+	÷	+						+
	Saponins	+	÷	÷	÷	÷			÷	+		÷	+	÷	+	
	Tannins	+	÷	÷	÷	÷	÷		÷	+		÷	+	÷	+	+
	Phenolic compounds	÷	+				+							+		+

(+) = Presence of the compound; (-) = Absence of the compound



Table 2

Extract yields for petroleum ether, acetone, and ethyl acetate solvents extracts for selected plants (trees, shrubs, herbs).

	Dianta		Plant part	Mass o	f extract yield (%)	
Sr. No.	Plants	Common name	used	Petroleum ether (PE)	Acetone (ACE)	Ethyl acetate (EA)
1	Alstonia scholaris	Saptaparni	Leaves	1.5	1.55	3.19
2	Floral waste	Rose,marigold, tuberose	Flowers	0.88	2.13	1.77
3	Azadirachta indica	Neem	Leaves	1.01	1.25	2.11
4	Moringa oliefera	Drumstick	Leaves	1.68	1	1.34
5	Plumera rubra	Frangipani	Leaves	1.46	1.57	0.14
6	Lantana camera	Wild Sage	Leaves	0.12	0.14	1.46
7	Calotropis gigantia	Giant milkweed	leaves	0.99	1.03	0.1
8	Anona squamosa	Sugar apple	Leaves	0.08	0.1	0.29
9	Cassia tora	Sickle pod	Leaves	1.35	0.23	0.2
10	Hibiscus rosa sinensis	Chinese hibiscus	Leaves	0.12	0.11	1.12
11	Psidium guava	Guava	Leaves	0.22	1.14	1.98
12	Cascabela thevetia	Yellow oleander	Leaves	1.74	0.22	0.05
13	Ocimum sanctum	tulsi	Leaves	0.06	0.12	0.2
14	Euphorbia hirta	Asthma weed	Leaves,	1.08	0.11	0.09
15	Ipomea purpurea	Common morning glory	Leaves	1.16	0.1	0.06







Fig. 1. Antibacterial activity of different green waste extracts; 1: *Azadirachta indica* ethyl acetate extract against *Staphylococcus aureus*; 2: *Euphorbia hirta* acetone extract against *Staphylococcus aureus*; 3: *Alstonia scholaris* ethyl acetate extract against *Pseudomonas aeruginosa*; 4: *Anona squamosa* petroleum ether extract against *Pseudomonas aeruginosa*; 5: Floral waste acetone extract against *Escherichia coli*; 6: *Ocimum sanctum* ethyl acetate extract against *Escherichia coli*.





Fig. 2. Antimicrobial activity of selected plants from acetone extract.



Fig. 3. Antimicrobial activity of selected plants from ethyl acetate extract.





Fig. 4. Antimicrobial activity of selected plants from petroleum ether extract.

different solvents (acetone, ethyl acetate, petroleum ether) delineated various degrees of antimicrobial activities. Still, the most promising results were detected for the ethyl acetate extracts of Alstonia, Floral waste, Azadirachta, Plumera, Euphorbia, etc., against P. aeruginosa, followed by the acetone extracts for all the bacterial species. Petroleum ether extracts were the least effective but revealed antibacterial activities against the selected strains. The plant parts that are being wasted or dumped can be used as natural products for these strains and can also be applied for the other bacterial strains and other micro-organisms that can be a threat to humans. The plant extracts can be further studied for the phytochemical analysis, which gives favourable yields. The studies will also provide a clear picture of the secondary metabolites or the bioactive compounds responsible for antimicrobial activity.

4. Concluding remarks

By the preliminary antimicrobial investigation, it can be concluded that given a scope of considerable research for the green waste, the phytochemicals present in it can probably set off as one of the potential alternatives to the synthetic drugs and multidrug-resistant pathogens as well as an innovative way to recycle reuse and reduce the waste generation in the environment. Plants consist of various degrees of inhibitory effects against the pathogens tested. The most significant antimicrobial activity was demonstrated by *Alstonia scholaris*, Floral waste, *Azadirachta indica, Euphorbia hirta*, etc., which depicts potential antibacterial components. Pharmaceutical industries can further investigate it for their active phytochemical ingredients against various diseases caused by these pathogens. Since the research is in its initial stages, the prospects can scrutinize the extraction of plants parts and lead to the antimicrobial tests against other gramnegative, gram-positive bacteria and fungus and the development of potential antimicrobial and antifungal products. The phytochemical analysis can be carried out in the future to isolate and separate the secondary metabolites, which can be separately produced as new drugs. By this method, the green and brown waste can also be managed and is an excellent option apart from composting, burning, and dumping to fulfil the sustainable development goals of reducing, reusing, and recycling waste generated in the environment. The studies carried out and discussed in the article are much at an initial phase or small-scale trials only; therefore, there is much need for research and development activities for the economic and sustainable development of these resources for environmentally sustainable ecoclean energy production at a colossal scale.

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Guidelines for the study of plants in the research

All the analyses were performed with the plants, which are the least concern according to IUCN and are part of yard waste. No harm was done to any plant parts which



are vulnerable or fall in other such categories.

Conflict of interest

The authors declare that there is no conflict of interest.

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