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RESEARCH ARTICLE

Identification of Bacterial Endophytes Isolated from Selected Medicinal Plants

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Abstract

Plants have very potential compounds to live on earth as they supply 90% of human calorie intake, 80% of protein intake directly, and perhaps the most vital sources of medicine with a vast diversity of microorganisms. As such it's important to know those microorganisms, their kinds, the features they possess, and the significant compounds/metabolites they can produce. So, this study is based on identifying such microorganisms. To achieve this aim, isolation of endophytes has been done to know their biochemical activities and properties. Various identification procedures have been followed to get pure endophytic strains without any contamination. Surface sterilization of the plant tissue is a must in this progress, various surface sterilization techniques have been tried and finally, for 4/5 plant tissues, sodium hypochlorite and ethanol were given the best result and for 1/5 with the addition of mercuric chloride were the standardized method for surface sterilization. About 30 different bacterial endophytes have been isolated from five kinds of medicinal plants. 4% sodium hypochlorite and 75% ethanol were found effective in sterilizing the surface of Psidium guajava, Cassia occidentalis, Calotropis procera, and Hibiscus rosa-sinensa. While Mangifera indica required an addition of 0.1% mercuric chloride. 19 strains isolated were Gram-positive, 11 Gram-negative (5 were Lactose fermenters and 6 were not), and most of which were bacilli. All isolates have shown different biochemical results, 25 showed a positive result for oxidase, and 28 gave a positive result for catalase. Most of the endophytes identified in this work are Bacillus spp. However, this study highlighted the significance of surface sterilisation and most importantly the presence of potential endophytes capable of producing novel bioactive compounds usable in pharmaceutical/ medicinal application.

Key Words: *Medicinal plants; Endophytes; Surface sterilization; Isolation; Identification*

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Introduction

Plants are known to be used as medicine for chiliastic. As the current knowledge interprets it, especially in Africa(Nigeria), it's known that extracts of these plants are used in the crude form as medicine, e.g. *Cassia occidentalis* for typhoid, *Calotropis procera* as an antifungal, etc. Concurrently, most of the drugs used presently are sourced from plants. Medicinal plants are the reservoir of secondary metabolites used in drug making and important oils that are potentially therapeutic. The primacy of medicinal plants for medications in various afflictions is their security, ease of accessibility, inexpensive, and effectiveness. These supremacies of the medicinal plants necessitate their extensive use daily by ethnobotanists. Plants are divergently inhabiting numerous environments interacting with the microbes present in their habitats. Incidentally, microbes and plants operate cooperatively as powerful bodies to effect primal resolution to the environmental challenges that led to the "holobiont" conception [1]. Plant-microbiome associations are divergent in nature and ubiquitary [2-4]. Endophytism is aquestion of history known to be a natural phenomenon originating seemingly long antecedes to the presence of plants on earth [5], advanced by De Barry as a discipline of science when he came up with the 4th concept of "Endophytes". The realization of the medicinal and ecological importance of this area of science recently attracted much curiosity [6] and resulted in a high scientific interest. The term endophyte was from Greek ('endon', within, and 'phyton', plant). Endophytes are microbes that are associated with plants and have the capability of dwelling within the plant tissues having no symptoms of infection or harm [7]. Endophytes involve divergent microbial groups, and there is a wide number of endophytes in association with most of the plants which are nonfungal. Mostly, Plant endophytes are bacteria and fungi. Whereas algae, archaebacteria, nematodes, and protozoa are difficultly found to be dwelled as endophytes; however, they enjoy significant results on the plant [8,9]. Bacterial endophytes residing within the plant organ abstain of being destructive or beneficiary but habitation. They are of a wide variety and naturally polyphyletic [10]. Their isolation can be succeeded from different parts of plants both above and below, tremendously in the roots. The rhizosphere is said to be the usual route of bacterial endophyte settlement [11]. Endophytes were recognized a long time ago as a good fount of bioactive compounds main for pharmaceutics, as many of them are known with significant ability to produce unique biologically active metabolites encompass antimicrobial (antibacterial, antifungal, and antiviral) as well as many related significant compounds. More so, endophytes have been demonstrated important in producing various classes of natural products and also exhibiting a broad range of activity in biological systems categorized into many classes. They are lactones, steroids, lignans, alkaloids, terpenoids, phenolic compounds, quinones, etc.Supremely, endophytes produce secondary metabolites that provide excellent fitness enhancements and a lot of benefits to the host plants, like fixation of nitrogen, plant growth stimulation, ability to resist drought, herbivorous, and parasitism [12].

Guava (*Psidium guajava* L.) is a medicinal tree native to South America producing portentous fruits, belong to family Myrtaceae. It's grown globally in many subtropical and tropical regions [13]. *Psidiumguajava* (bark, fruits, and leaves) has a long history of ethnobotanical significant for curing and prevention of diseases in many countries [14,15]. Currently, the leaves help in treatment of cough, cold, laxatives, wounds, vomiting, dysentery, diarrhea, hyperglycemia, and gastrointestinal disorders [16,17]. So many reports have proven the momentous features of Guava leave as anti-diabetic [18,19], antioxidant [20,21], antibacterial effects [22,23], presence of anti-inflammatory substance [24,25], having antiparasitic compounds [25], anticancer activity [25] and hepatoprotective activity. More so, guava extracts could be used as

Immunomodulators [26], antiviral [27] and certainty of having inotropic compounds [28]. The fruits are mostly used freshly as food and in making juices containing very high mineral and vitamin content. It's considered among the fruits with high nutritionalvalue resulted a wide diversification in purpose.

Mango tree (*Mangifera indica* L.) came into existence 4000yrs back in India, included among the most widely distributed trees in South-East Asia. Its gained substantial adoration worldwide for its peculiar taste varying from sour to sweet, aroma, and being a freshening food in summertime in equatorials. It's used traditionally as natural and renewable source of preparing medicinal formulation [29,30]. Suitably, mango tree parts such as leaves, bark, root, peels, kernel, flowers, pulp, twigs, branches, raw and ripe and unripe fruits, can be purposely used for variable therapeutic and dietetic needs. Evidence have appeared in so many reports that mango tree and fruits contain multiple structures of pharmaceutical bioactive compounds and chemical constituents. They include proteins, fats, polyalcohols like (xylitol, sorbitol, and myoinositol); significant peculiar carbohydrates (glucose, galactose, and arabinose); fatty acids, alkaloids, volatile substances, and saponins (estearic, linoleic, oleic, myristic and palmitic acids); (polyunsaturated and dicarboxylic acid); vitamin B (niacin, riboflavin, and thiamine), vitamins C, E, and ß- carotene, are needed minerals and vitamins (calcium, iron, magnesium, manganese, potassium, zinc, copper, hydrolyzable tannins, and gallic tannins); anthocyanidins, bioflavonoids, polyphenols, leucoanthocyanins, (delphinidin, cyanidin, peonidin, and gallic tannins); and lastly mangiferin, with tremendous distribution in fruits, leaves or throughout the entire tree [31-34].

Cassia occidentalis L. commonly known as Coffee senna, fetid cassia, and Negro Coffee (English). It's an annual or perennial weed with a long ethnobotanical history considered as among the highly potential Ayurvedic plants which is used in several traditional medicines to cure various diseases. *C. occdentalis* is found abundantly grown in both tropical and subtropical regions throughout the world [35]. Large number of research had reported several important chemical presences in *C. occidentalis* some of which are achrosin, aloe-emodin, emodin, anthraquinones, chrysoberyl, emodin, essential oils, funiculosin, galactopyranosyl, helminthosporine, quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorine [36-39] reported antimicrobial activity of C. occidentelis against different microbes. Moreso, it possesses antimalarial activity [40,41], antioxidant [42], anti-inflammatory [43] anticancerous, antimutagenic, and immunostimulant activity [44,45].

Calotropis procera (Aiton) is a perpetual shallow tree grown in numerous desert and sub-desert regional countries, including tropical and subtropical continents. [46] reported high socioeconomic value of C. *Procera* in Latin America. Also to be found abundantly in Middle east, Africa, and Asia [47,48] *C. procera* has diverse common names. They are the Calotrope cabbage tree, Giant milkweed, Madar, Rubber tree, and Sodom apple, [49,50]. There are various significant chemicals affirmed presence in *C. procera*. Some of these are phytosterols, triterpenoids, and Pentacyclic triterpenes in the roots [51]. Amyrin acetate ß-sitosterol and ursolic within the leaves [52]. While caoutchouc, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin, and proceroside are presence in the latex [51,53]. reported the potentiality of C. *procera* as a good phytoremediation instrument with higher Pb and Cd accumulation. C. procera is known to be used for the treatment of burn injuries and also serves as an antidote for mumps and rheumatism [54].

Hibiscus rosa-sinensis L. is an ornamental, perennial woody, and therapeutic flowering plant grouped under the family Malvaceae. Its extensively dispersed in equatorial regions. It has a history of being used as a medicinal

constituent for several years ago. There are reports that indicated *H. rosa-sinensis* is a good conductor of bioactive compounds, which certified it could reliably serve to relief and cure lots of diseases therapeutically [55]. *Hibiscus rosa-sinensis* has indicated significant features of antioxidant and antimicrobial activities [56].

Endophytes are the substitutional way of accessing secondary metabolites that are biologically active, the need to improve the microbial fermentation process arises here. Mainly, to increase the mass production of active biological composites, rather than to use copious plant materials which can lead to deforestation, decreased biodiversity, and conservational loss [57,58]. The aim of this work is to isolate, characterize, and identify new endophytic species from plants. And it's crucial as highlighted by [12].

Methods

Collection of plant sample materials

Psidium guajava, Mangifera indica, Cassia occidentalis, Calotropis procera, and Hibiscus rosa-sinensa healthy parts, namely, to be leaves, roots, stem, bark, and flowers were selected and randomly collected at different locations from Parul Institute of applied science's and University Botanical/medicinal garden. The samples collected were carried separately inside clean sterile plastic bags and quickly brought to the laboratory to maintain their freshness. Which were immediately used for the experimental work for aimed examination.

Plant sample purification

This could be achieved by treating the plant tissues with a general sterilizing agent or oxidant at diverse times and followed with 2-5 multiple sterilized rinsing [59]. However, it's expected that the medium used for the sterilization can eradicate the entire microbes to be found on the surface of the plant by not damaging the endophytes and the host tissue [59-61]. This makes the step somehow critical as some of the reagents might destroy the endophytes considering the duration needed to extinguish the final epiphyte on the plant part surface.

Pre-treatment

The healthy tissues/organs of the five different plants collected which are flower, leaves, roots, stem, and back were washed independently under running tap water to clear away attached soil particles, waxes, and most epiphytes. Normally roots do not need extra pre-treatment, because their surfaces are free of hydrophobic substances such as leaf waxes. Brushing softly and washing could be sufficient [59].

Surface sterilization

At first, the freshly collected barks, flowers, leaves, roots, and stems are washed under tap water for 10-15 minutes and washed in Tween 20 (a drop in 200 mL SDW) come after for 1 minute. The plant tissues were transferred into a laminar air flow cabinet and rinsed three times with SDW. Commonly used sterilizing agents are ethanol 70-95% for 30 seconds to 4 minutes [62] and hydrogen peroxide 0.05 to 02% [63], extra careful has to be taken exclusively when using mercuric chloride 0.02 to 0.2% for plant tissues that require this agent to achieved sterility, due to heath reason [64] and sodium hypochlorite 1 to 5% for 2 to 10 minutes [65].

Sterility check and control

The microorganisms isolated are said to be considerably endophytes if only the maximum sterility of the plant tissue surface is achieved successfully. To authenticate the surface sterilization procedure there are three measures to contemplate: imprinting the sterilized surface of the plant tissue on nutrient media [66], aliquot growing of water from the final rinsing on nutrient media [67], submerging the surface sterilized explant in the nutrient broth [68] and lastly to be incubated representing control of the experimental sample. The NA plate with no inoculation of plant material for sterility check

serve as control.

The media used for endophytic bacteria Isolation

Medium is what determines the type and number of endophytic microorganisms to be isolated from different plant tissues, so the choice of the medium is very important. The media that goes well with bacterial endophyte isolation is nutrient agar (NA). Among other media that can be used to isolate bacterial endophytes are TSA and R2A (tryptic soya agar supporting the growth of bacteria with extensive range, while R2A supports the growth of bacteria that require low nutrient levels [69].

Supplements

There are supplements that are added to the media to suppress undesired or stimulate the likable growth of the microbiomes. The commonly used are antibiotics, fungicides like nystatin, or specific nutrients [60].

Purification, isolation, and sub-culturing of bacterial endophytes

Following the final rinsing of the sterilized plant tissue surfaces in the laminar air flow, aseptically the surface of the stems was removed using a sterile scalpel, leaves were cut into pieces, so also the flower and the roots. The pieces were dried properly after which the pieces were implanted upon the nutrient agar plate. Each plate was independently inoculated with 2-3 small pieces of the plant organ and was incubated at 37°C to redeem the possible bacterial endophyte colonies maximally. The plates were observed between the period of 24 to 48 hours. Morphologically, the colonies of the different bacteria observed were sub-cultured and streaked many times on nutrient agar plates to attain pure bacterial isolates. Lastly, the clarified endophytes were kept at 4°C for use mean.

Bacterial endophytes characterization

Phenotypically the microscopic features, gram staining, biochemical tests, catalase, oxidase, lactose, and Triple Sugar Iron activity of the entire isolated strains were noted as the results expressed with the aid of mirroring standard procedures.

Different sterilization treatments to samples

Getting the excellent surface sterilization condition of the five different medicinal plants used in this work requires an employing of different sterilizing agents accurately combined as its detailed in Table 1. Three experiments were repeatedlydone, and the data represents the mean of the three experiments. After statistical analysis, the efficacy of sterilizing agents with an effective combination was seen, and various results were noticed in five different medicinal plants. These results were subjected to in the form of percentage.

Results

Isolation procedure and purification

Surface sterilisation

Dual treatment of 75% Ethanol for 45 seconds and 4% Sodium hypochlorite for 1 minutes was used in sterilising leaves, stem, bark and flower of *Psidium guajava, Cassia occidentalis*, *Calotropis procera* and *Hibiscus rosa-sinensa* (Table 1). For *Cassia occidenatalis* root an extension to 1 minutes for 75% Ethanol and 1 min, 30 secs for 4% Sodium hypochlorite were required. And *Mangifera indica* leaves and bark was sterilised with 75% ethanol 45 seconds, 4% Sodium hypochlorite for 1 minute and 0.1% mercuric chloride for 30 seconds (Table 1).

The effect of mercuric chloride, ethanol and sodium hypochlorite used was observed in order to know their efficiency as sterilizing agents. The initial procedures selected for surface sterilisation were not found suitable independently as sterilising agent. Due to that, the percentage of explant contamination was found high. Moreover, a severe damage was seen on the explants were seen when 0.1% mercuric chloride was used in combination with 75%

Ethanol, as well as Sodium hypochlorite except in the treatments of *Mangifera indica* (Fig 2).

Subsequently, different duration and combination of 75% Ethanol and 4% Sodium hypochlorite gives satisfactory results as seen in (Figs 1,3,4 and 5) resulted to a high survival percentage.

Sterility check

Surface sterilisation was effectively achieved because there was no microbial growth appeared on the control plate upon growing aliquot of water from the final rinsing on nutrient media plate.

Purification and isolation

Nutrient agar was the media used for successful isolation of the bacterial endophyte in this work. Several colonies of different kinds were recovered after an optimal incubation. 30 strains of bacteria had been purely isolated from five medicinal plants used as samples of this study; the details are expressed in Fig. 6. Growth of purified bacterial endophyte on the stem of *C. procera* and leaves of *P. guajava* cultured are shown in Fig. 7. Streaked pure culture obtained from the stem of *C. procera* and leaves of *P. guajava* shown in Fig. 8.

Identification of endophytic bacteria

Isolated endophytic bacteria possessed varied morphological characteristics as observed. There are multiple colonies, almost all the isolates are rod-shaped with irregular and round colonies. Some off-white, yellow, and orange (Fig.7). Concerning their phenotypic feature: 19 gram-positive bacilli and 11 were seen to be gram-negative bacilli (Table 2). For biochemical and physiological tests, 5 were Lactose fermenters and 6 were not, most of which were bacilli. All isolates have shown different biochemical results, 25 showed positive results for oxidase, and 28 gave positive catalase, they also possessed variable TSI results with a lot of them Y/Y, Y/R, and R/Y only a few were affirmed to produce gas, and none was capable of producing H2S gas as illustrated in Table 2. 17. The endophytes were identified as *Bacillus spp,* 4 are *Pseudomonas spp,* 2 are *Achromobacter spp*, 2 are *Enterobacter spp,* and *Escherichia coli, Coccobacilli/streptococcus*, *Klebsiella spp, Citrobacter spp,* and *Siccibacter colletis* are representing one isolates each (Table 2).

TABLE 1

Variable duration, combination, and concentration of treatment used for sterilization.

Sterilizing (treatment)	Plant organ/Explant	Treatment Period
75% ethanol (X)	Flower, Stem, leaves,roots	45 seconds 1 minute
4% sodium hypochlorite(Y)	Flower, Stem, leaves, roots	1 minute 1 min, 30sec
0.1% mercuric chloride(Z)	Flower, Stem, leaves, roots	30 seconds
75% ethanol + 4% sodium hypochlorite(X+Y)	Flower, Stem, leaves, androots	45 secs + 1 minute 1 min + 1 min, 30 secs
75% ethanol+ 0.1% mercuric chloride(X+Z)	Flower, Stem, leaves, roots	45 sec + 30 secs 1 min + 30secs
4% sodium hypochlorite +0.1% mercuric chloride (Y+Z)	Flower, Stem, leaves,roots	1 min + 30 secs 1min, 30secs + 30secs
75% ethanol + 4% sodium hypochlorite + 0.1% mercuric chloride(X+Y+Z)	Flower, Stem, leaves, Root	45secs + 1min + 30secs 1min + 1min, 30secs + 30secs

TABLE 2
Biochemical characteristics of the isolated endophytic bacteria

Isolates	G Stn	Ind Tst	MR Test	VP Tst	Ci Tst	Ca Tst	Oxi Tst	Lac Tst	TSI Medium				Species
									Slnt	But	H2S	Gas	
GL1	-	-	-	-	-	+	+	NLF	Y	Y	-	-	*Achromobacter xylosoxidase*
GL2	+	-	+	+	-	+	-	GP	R	R	-	-	*Bacillus spp*
GL3	+	-	+	-	-	+	+	GP	Y	Y	-	-	*Bacillus megaterium*
GL4	+	-	+	+	-	+	+	GP	Y	R	-	-	*Bacillus cereus*
GL5	+	-	+	+	-	+	-	GP	R	Y	-	-	*Bacillus pacificus*
GB1	+	-	+	+	+	-	+	GP	Y	Y	-	-	*Bacillus cereus*
GB2	-	-	+	+	+	+	+	LF	R	Y	-	-	*Pseudomonas chlororaphis*
GB3	+	-	-	-	-	-	+	GP	Y	Y	-	+	*Bacillus amyloliquefaciens*
GST1	-	-	+	-	-	+	+	NLF	Y	Y	-	+	*Citrobacter koseri*
ML1	+	-	+	-	-	+	-	GP	R/Y	Y	-	-	*Bacillus spp.*
ML2	+	-	+	+	-	+	+	GP	R	Y	-	-	*Bacillus amyloliquefaciens*
MB1	+	-	-	-	-	+	+	GP	Y	Y	-	-	*Bacillus mojavensis*
MB2	+	-	+	+	-	+	+	GP	R	Y	-	-	*Bacillus subtilis*
MB3	+	-	+	+	+	+	+	GP	R	Y	-	-	*Bacillus pumilus*
COL1	+	-	+	-	-	+	+	GP	Y	Y	-	-	*Bacillus amyloliquefaciens*
COL2	+	-	+	+	-	+	+	GP	R	Y	-	-	*Bacillus pumilus*
COL3	-	-	+	+	+	+	-	LF	R	R	-	-	*Klebsiella terrigina*
COB1	-	-	+	-	-	+	+	LF	Y	Y	-	-	*Siccibacter colletis*
COB2	+	-	+	+	-	+	-	GP	R	Y	-	-	*Bacillus anthraciis*
COR1	-	+	+	+	-	+	+	NLF	R	Y	-	-	*Pseudomonas putida*
COR2	+	-	+	+	-	+	+	GP	Y	Y	-	-	*Bacillus cereus*
CPL1	-	-	+	-	+	+	+	NLF	Y	Y	-	-	*Pseudomonas gramnia*
CPF1	+	-	-	+	+	+	+	GP	R	Y	-	-	*E aerogenes*
CPF2	-	+	+	+	-	-	+	GP	Y	Y	-	+	*Escherichia coli*
CPS1	+	-	+	+	-	+	+	GP	Y	Y	-	-	*Bacillus oleronius*
CPS2	-	-	+	-	-	+	+	NLF	Y	Y	-	-	*Pseudomonas chlororaphis*
HBL1	+	-	-	-	-	+	+	GP	Y	Y	-	-	*Achromobacter xylosoxidans*
HBL2	+	-	+	+	+	+	+	GP	Y	Y	-	-	*Bacillus pumilus*
HBL3	-	-	+	-	+	-	+	NLF	Y	Y	-	-	*Coccobacilli/streptococcus*
HBST1	-	-	+	+	+	+	+	LF	Y	Y	-	-	*Enterobacter spp*

Codes regarding origin GL: *Psidium guajava* leaf, GB: *Psidium guajava* bark, GS: *Psidium guajava* stem, , ML: *Mangifera indIca* leaf, MB: *Mangifera indIca* bark, COL*: Cassia occidentalis* leaf, COB: *Cassia occidentalis* bark, COR: *Cassia occidentalis* root, CPL: *Calotropis procera* leaf, CPS: *Calotropis procera* stem, HBL: *Hibiscus rosa-sinensa* leaf, HBST: *Hibiscus rosa-sinensa* and stem and isolates number is indicated as 1, 2, 3 4 and 5.

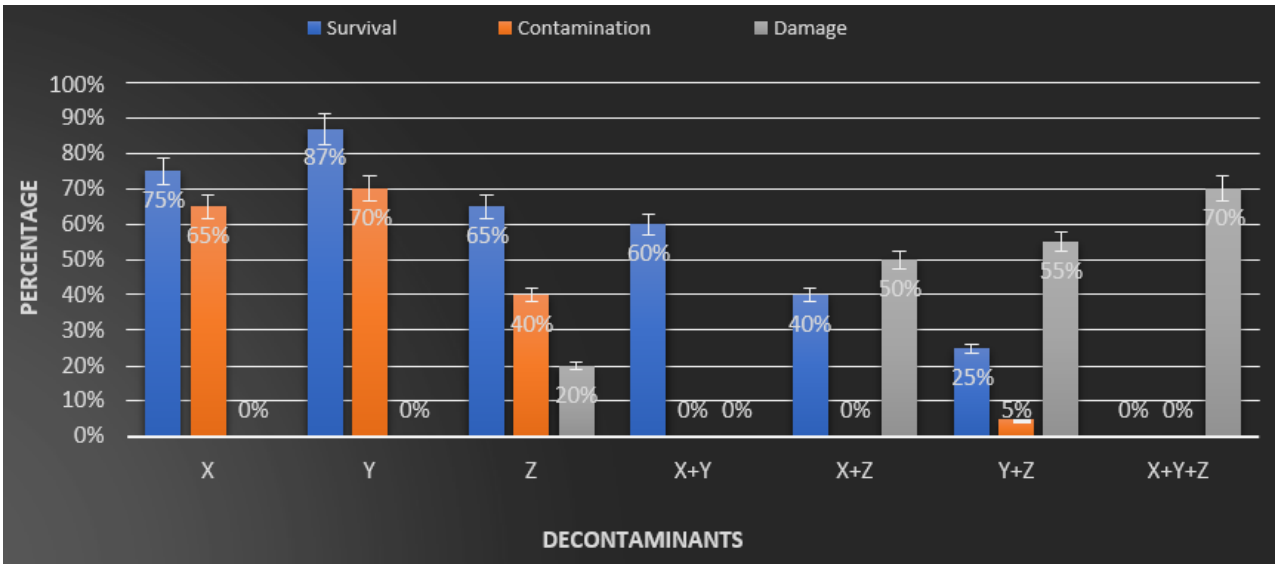

Figure 1) *Psidium guajava surface sterilization*

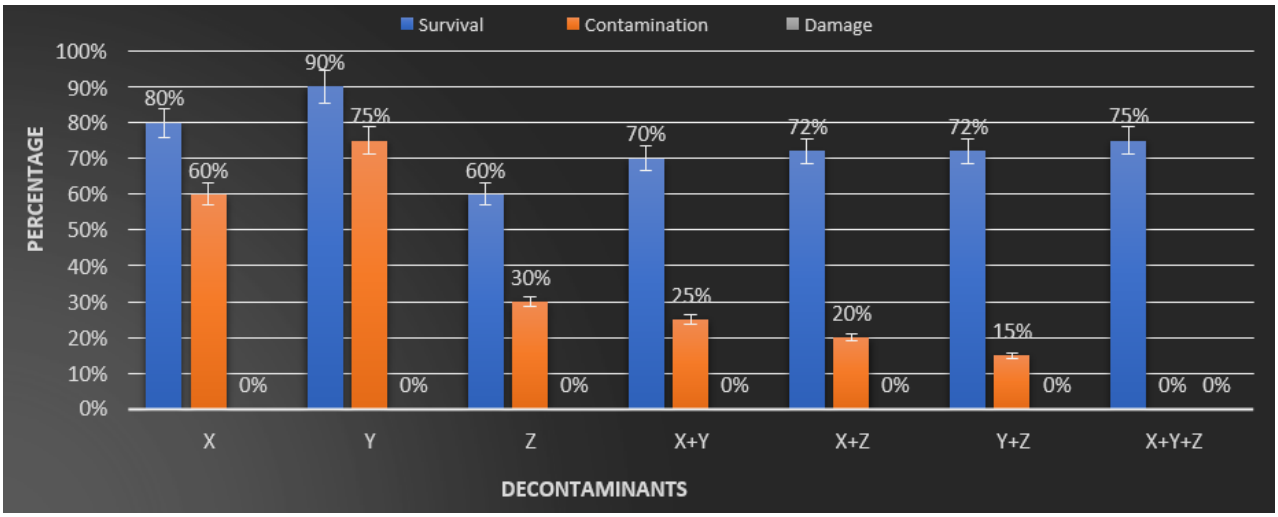

Figure 2) *Mangifera indica surface sterilization*

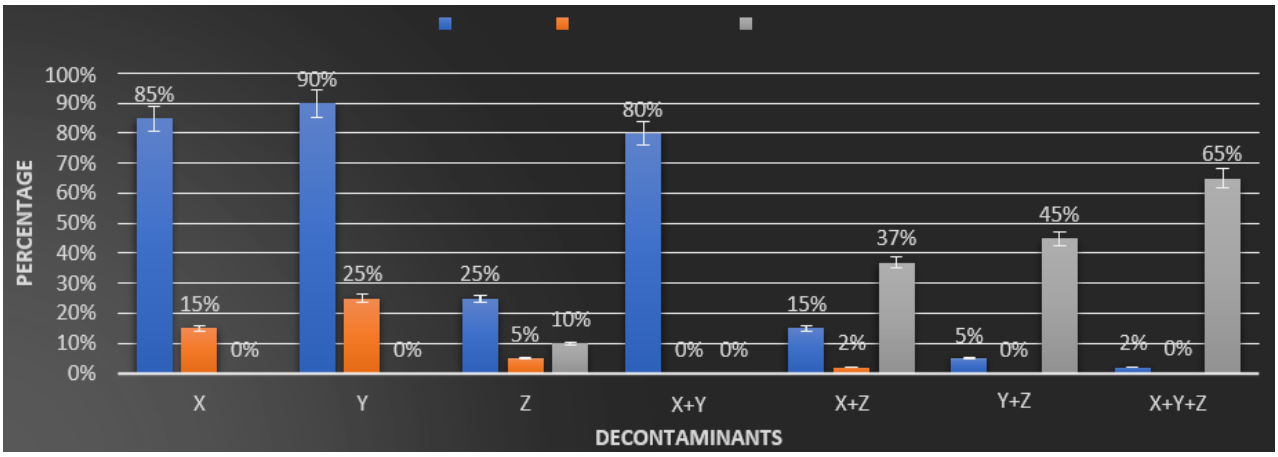

Figure 3) *Cassia occidentalis surface sterilization*

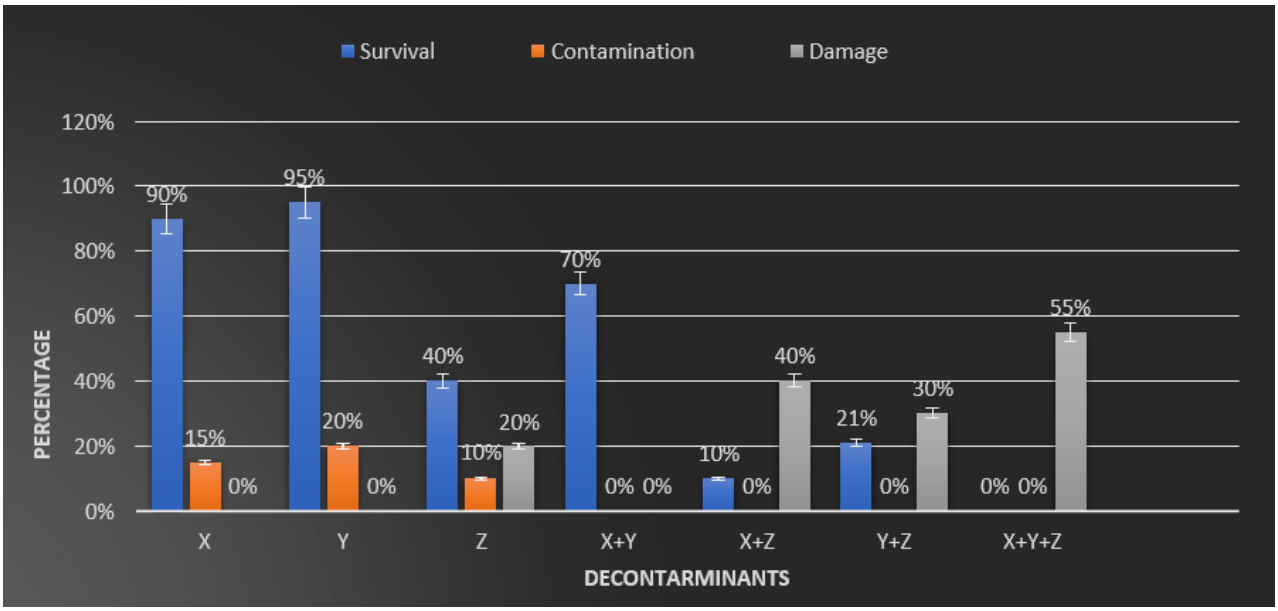

Figure 4) *Calotropis procera surface sterilization*

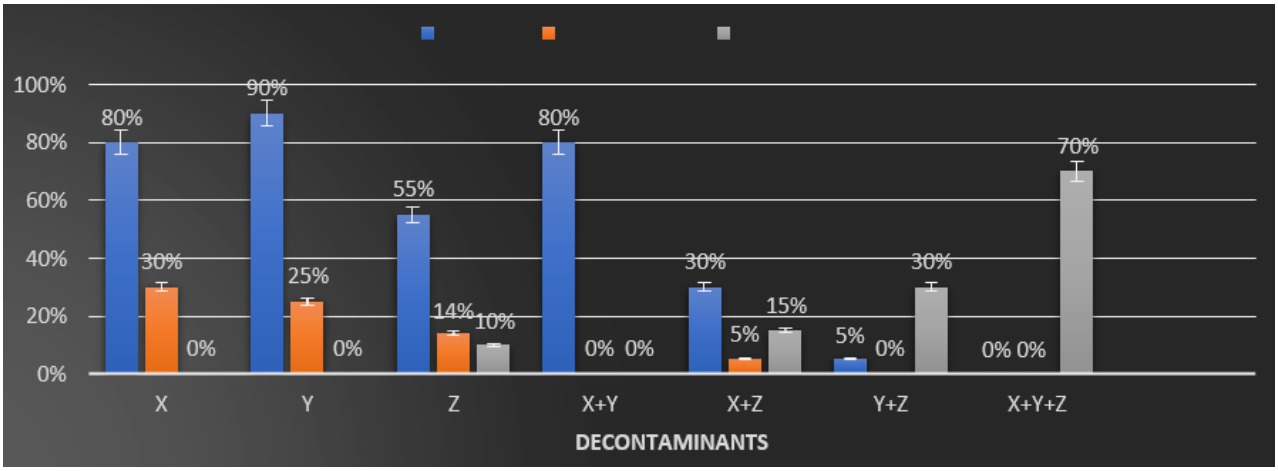

Figure 5) *Hibiscus rosa-sinensa surface sterilization*

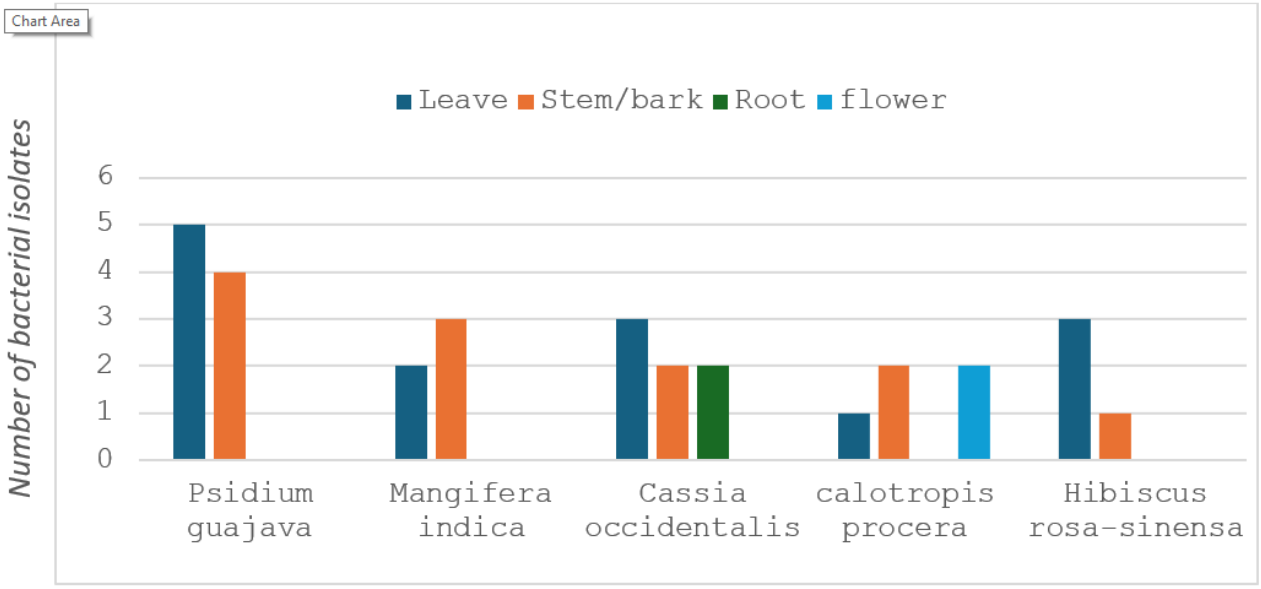

Figure 6) *Endophytic bacterial isolates in numbers from five medicinal plants*

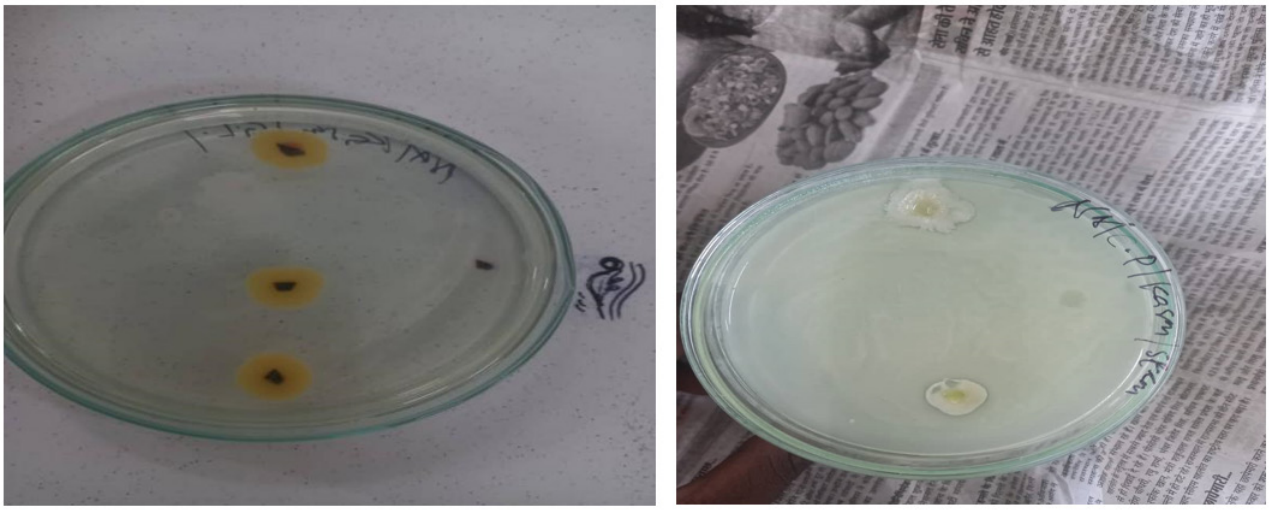

Figure 7) *Bacterial growth on the leaves samples as endophytes of P. guajava (L) and stem of C. procera (R)*

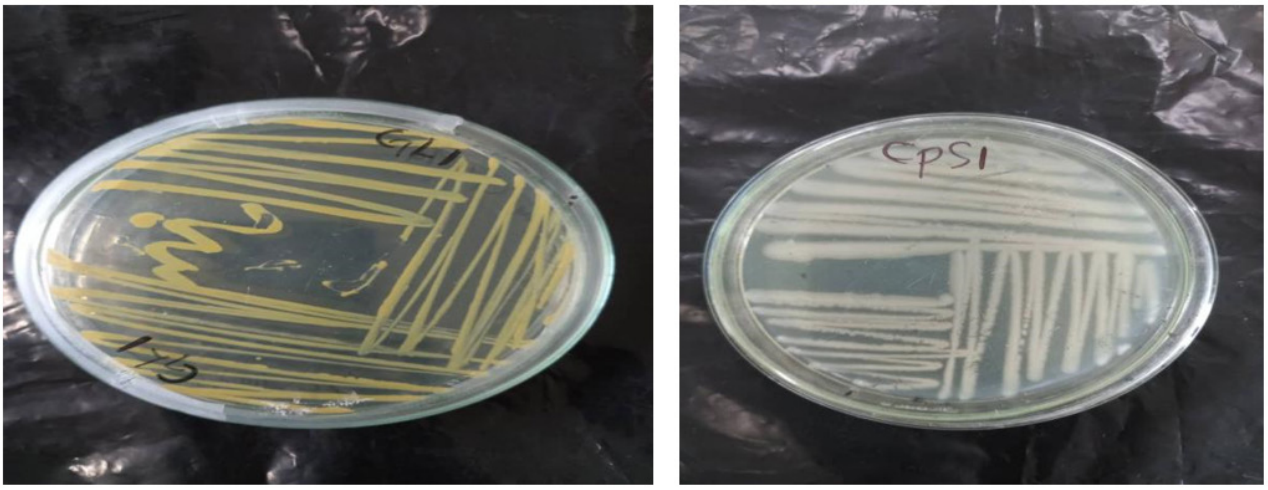

Figure 8) *Pure culture of bacteria Streaked from (L) P. guajava leaves and (R) C. procera stem*

Identified bacteria and their region of isolation

The bacterial endophytes isolated were identified based on the biochemical, morphological and physiological features they possessed. 9 strains were isolated from *Psidium guajava* five of which are from the leaves and they were identified as GL-1 (*Archromobacter xylosoxidase*), GL-2 (*Bacillus spp*). GL-3 (*Bacillus megaterium*), GL-4 (Bacillus *cereus*), and GL-5 (*Bacillus pacificus*), those isolated from the back are identified as GB-1 (*Bacillus cereus*), GB-2 (*Pseudomonas chlororaphis*) and GB-3 (*Bacillus amyloliquefaciens*), only one was isolated from stem as GST-1 and identified *Citrobacter koseri* (Table 2). The isolates of *Mangifera indica* includes ML-1 (*Bacillus spp*), ML-2 (*Bacillus amyloliquefaciens*) from the leaves but MB-1(*Bacillus mojavensis*), MB-2 (*Bacillus subtilis*) and MB-3 (*Bacillus pumilus*) were isolated from the bark (Table 2). COL-1 (*Bacillus amyloliquefaciens*), COL-2 (*Bacillus pumilus*) and COL-3 (*Klebsiella terrigina*) are from *Cassia occidendatalis* leaves while COB-1 (*Siccibacter colletis*) and COB-2 (*Bacillus anthraciis*) from the bark of *Cassia occidendatalis*, COR-1 (*Pseudomonas putida*) and COR-2 (*Bacillus cereus*) were isolated from *Cassia occidendatalis* root (Table 2). *Calotropis procera* has only one isolate from leaves as CPL-1 (*Pseudomonas gramnia*) whereas CPF-1 (*E aerogenes*) and CPF-2 (*Escherichia coli*) are from flower, CPS-1 (*Bacillus oleronius*) and CPS-2 (*Pseudomonas chlororaphis*) were isolated from *Calotropis procera* stem (Table 2). *Hibiscus rosa-sinensa* has the lowest

number of isolates HBL-1 (*Archromobacter xylosixidans*), HBL-2 (*Bacillus pumilus*) and HBL-3 (*Coccobacilli*) were from the leaves while HBST-1 (*Enterobacter spp*) was from the stem (Table 2).

Discussion

Surface sterilization is so crucial as basic step to be taken for successful isolation of endophytes. Different decontaminants were employed with a proper monitoring (Table 1). The favorable condition for the surface sterilization that yielded desired survival of the tissues from *Psidium guajava, Cassia occidentalis, Calotropis procera, and Hibiscus rosa-sinensa* was by invigorating the leaves, flower, and stems using 75% ethanol for 45 seconds, 4% sodium hypochlorite for 1 minute while 1 minute 30 seconds for root, respectively (Fig. 1,3,4 & 5). Whereas condition suitable to disinfect *Mangifera indica* was by sanitizing the stem and leave with 75% ethanol for 1 minute, 4% sodium hypochlorite for 1 minute 30 seconds and 0.1% mercuric chloride for 30 seconds (Fig. 2). The application of mercuric chloride for sterilizing the surface of *Psidium guajava, Cassia occidentalis, Calotropis procera* and *Hibiscus rosa-sinens* was ineffective because it destroyed the explants even though it's good decontaminant but toxic. [62] had reported many sterilants including ethanol (70%–90%), sodium hypochlorite (2%–10%), and mercuric chloride (0.1%). 70% ethanol for 3 minutes, 0.5% sodium chlorite for 3 mins, and 70% ethanol for 3 mins were also reported as the only decontaminants used in isolation of bacterial endophytes from *Curcuma longa* L [70].

A total of 30 different bacterial strains were isolated. Out of which 9 are from *Psidium guajava,* leaves (5), bark (3) and stem (1). [71] reported 7 fungal endophytes from *Psidium guajava* five of which are *Alternaria* sp. and two are *Fusarium* sp. Another report by [72] highlighted *Fusarium* sp. and *Cladosporium* sp. as endophytic fungi isolated from the leaves of *Psidium guajava* with great antimicrobial potentiality. Among the few reports on bacterial endophytes from *Psidium guajava* are *Streptococcus* sp., *Staphylococcus albus* and *Staphylococcus seiuri* [73], *K. quasivaricola, B. cereus, B. amyloliquifaciens, P. aureoginosa, B. subtilis, B. altitudinis, B. megaterium* [74] which is the recent report. We isolated *Achromobacter xylosoxidase, Bacillus* sp*., Bacillus megaterium, Bacillus cereus, Bacillus pacificus, Pseudomonas chlororaphis, Bacillus amyloliquefaciens,* and *Citrobacter koseri* from *Psidium guajava* as well. Some of our isolates goes in hand with few strains isolated by [74], we could be the first to *Achromobacter xylosoxidase, Bacillus* sp*., Bacillus pacificus, Pseudomonas chlororaphis*, and *Citrobacter koseri* as bacterial endophytes from *Psidium guajava.* 7 isolates (*Bacillus amyloliquefaciens, Bacillus pumilus, Klebsiella terrigina, Siccibacter colletis, Bacillus anthraciis, Pseudomonas putida,* and *Bacillus cereus*) were isolated from *Cassia occidentalis.* [75] reported *Bacillus subtilis, Bacillus* sp., *Agrobacterium tumefaciens, Pseudomonas* sp., and *Pseudomonas putida* as bacterial endophytes isolated from *Cassia tora* L. Furthermore, *Acidomonas, Asaia, Gluconobacter, Acetobacter, Neoasaia, Gluconacetobacter, Kozakia, Saccharibacter, Swaminathania, Tanticharoenia,* and *Granulibacter* were among the bacterial species reported to had been isolated from *Mangifera indica* as endophytes [76]. In contrary we revealed *Bacillus* sp., *Bacillus amyloliquefaciens, Bacillus mojavensis, Bacillus subtilis,* and *Bacillus pumilus* as the bacterial endophytes isolated from *Mangifera indica.* [73] report also highlighted *Staphylococcus xylulose*, *Staphylococcus intermedius*, and *Staphylococcus aureus* as endophytic bacteria isolated from *Mangifera indica*. 15 species of *Arcopilus, Humicola wallefii,* and *Dichotomopilus funicola* were reported as endophytic fungi isolated from *Mangifera*

indica [77]. In view of the endophytes isolated from *Calotropis procera,* [78] reported 12 bacterial endophytes named as R1, R2, R3, R4, R5, R6, L1, L2, L3, S1, S2, and S3 indicating 6 were isolated from the roots, 3 from the leaves and 3 from the stem all of which were not identified. Another bacterial endophytes were reported as gram positive bacilli (CPGPB), gram negative bacilli (CPGNB), and gram positive cocci (CPGPC) isolated from *C. procera* [79]. [80] reported *B. firmus* (Cpl1), *B. subtilis* subsp. *spizizenii* (Cpl13), *B. amyloliquefaciens* (Cpl10), *B. niabensis* (Cpl3), *B. subtilis* (Cpl4) from leaves and *Bacillus cereus* (Cps1) *Citricoccus alkalitolerans* (Cps2), *B. pumilus* (Cps3) from stem as identified bacterial endophytes from *C. procera*. We isolated 5 strains i.e (*Pseudomonas gramnia*) from leaves, (*E aerogenes, Escherichia coli*) from flower and (*Bacillus oleronius, Pseudomonas chlororaphis*) from stem all of which were identified and we might be the first to report endophytic bacteria from flower of *C. procera*. The works reported on endophytic bacteria from *H. rosa-sinensis* are few also, most of which the endophytes were not all identified. [81] isolated 8 endophytic bacteria (Hib-3, Hib-4, Hib-7, Hib-14, Hib-17, Hib-19, Hib-20, Hib-24), only Hib-3 was identified as *Pseudomonas oryzihabitans* due to its potent features of producing maximum asparaginase. [82] also reported 3 unidentified endophytic bacteria from *Hibiscus rosa-sinensis* as H1, H3 (Gram positive bacilli) and H2 (Gram positive coccobacilli). In our we reported 4 strains isolated from *Hibiscus rosa-sinensis* identified as (*Achromobacter xylosoxidans, Bacillus pumilus, Coccobacilli/ streptococcus*) from leaves and (*Enterobacter* sp.). to our knowledge our isolates could be first to be reported as endophytic bacteria from *Hibiscus rosa-sinensis.* Moreover, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus* sp., *Bacillus pumilis*, and *Pseudomonas putida* were also reported as bacterial endophytes isolated from *Curcuma longa* L. [70] which tallied with most of our isolates in general.

It's a clear indication of how beneficial our results could be as tendency of the isolates to produce many bioactive compounds with multiple functionalities is very high. All the isolates were affirmed to be endophytes based on the techniques used in their isolation. Consequently, the isolated strains could be ideal as it was revealed that endophytes are best to be recognised as a depot for unique metabolites of attainable pharmaceutical, industrial and pharmaceutical importance. Thus, they are newly prospective sources of functionalized molecules significantly as vehicles for biotechnological operations [83]. As consequence of these characters, endophytes are paramountcy in perspective of bioprospection achievable by biotechnological means with possibility of developing highly economical products from them [84]. The need for more research on bacterial endophytes specifically is cardinal due to plenteousness of plants in both terrestrial and aquatic habitat globally as all plants harboured potential microorganisms. Continuous research in this field should always be encouraged and attractive. Meanwhile, it could lead to discovering new supremacy bioactive compounds employable in pharmaceutical industries, and medicine. The identified endophytes in this work could be a good source of metabolites applicable in medicine as anticancer, antimicrobial, antioxidant, antidiabetic as well insecticide with several targets in humans, plants and animals.

Conclusion

This work could conclude that 75% ethanol and 4% sodium hypochlorite are good decontaminants specifically when combined. And 0.1% mercuric chloride is also good but too effective and strong. However, it also affirmed the presence of endophytic bacteria within the medicinal plants used in this work. As already confirmed, endophytes have significant features of being

utilized as tools for the production of enzymes and secondary metabolites they are capable of in excess. This makes them more advantageous being monitorable, less costly, and very easy to handle. Having affirmed the gravity of the ethnomedicinal plants chosen for this research work, further study on some important enzymes that the isolates could produce is going as well as identifying those with antioxidant and antimicrobial activity. Antidiabetic, anticancer and anti-inflammatory test are advised to be further performed to make sure high exploitation of the identified bacterial endophytes is attained.

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Conflict of Interest

Authors declare that there is no conflict of Interest.

Data Availability Statement

The above authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

The data were generated **Kasim Muhtari1,** that support the findings of this study are available on request from the corresponding author **Inampudi Sailaja2*** on request.

Statement of Experimental Research

Our Experimental research and field studies on plants were on both cultivated & wild plants, including the collection of plant material, with relevant institutional, national, and international guidelines and legislation importance were included in the study.

List of plants used in the study were

CULTIVATED	WILD
Psidium guajava, Mangifera indica,	*Cassia occidentalis, Calotropis procera, and Hibiscus rosa-sinensa*

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