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Research Article

# Green Chemistry: Phyto-antibiotics, A Green Antibiotics, Isolated from Medicinal Plant of West Bengal Targeting Multi-drug Resistant Bacteria

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## Abstract

Today green chemistry approaches have been introduced to reduced toxicity and environmental pollution. Interestingly, use of huge synthetic antibiotics caused generation of drug resistant bacteria with increased generation and amplification of mdr genes (bla, amp, tet, str, aph, aac, cat) and transposons (IS5, IS30, IS110 etc) in large plasmids. We coined the green antibiotics word to introduce phyto-chemicals that inhibited bacteria with specificity similar to synthetic drugs. Ideally plants secret anti-metabolites to retard the growth of microorganisms and thus a good source for new drug development. We explored the isolation and characterization of six organic plant extracts from medicinal plants of West Bengal. In truth, Taxol, Artiminisin, Etoposide and Camptothecin are well documented phyto-drugs used as anti-cancer and anti-parasitic drugs.

**Keywords:** Green Antibiotics; Heterogeneous Phyto-antibiotics; MDR-TB; MDR-Typhoid; Corona Virus Infection; Indian Medicinal Plants

## Introduction

Green chemistry is an area of chemistry and chemical engineering to develop drugs and industrial chemicals avoiding pollution [1-4]. Further, green revolution like green energy, green housing, green food are the areas where world adapting to save this world with its 800 millions peoples. Recently, herbal drugs have given priority against chemical synthesis of antibiotics and steroids which are very toxic to human and animal health with creation of multi-drug resistant bacteria as well as many metabolic syndromes and cancer [5,6]. Here, we refer green chemistry as herbal products and is very similar to ancient Indian ayurvedic crude medicine but now has purified and characterized. Recently, such phyto-chemicals structure and function have been characterized by HPLC, UV-Vis, NMR, FT-IR, GC-MASS, 2-D Gel, GelDoc, RT-PCR, and Transcriptosome technologies as well as Nano-technologies [7]. In reality, simple phyto-chemicals were purified by gel filtration, TLC and HPLC where such 95% pure chemicals were as good as synthetic antibiotics although any such antibiotic amy contains multiple related chemical structures. So, there was no chemical synthesis and no by-product toxic-chemicals generation, and phyto-extracts are mostly non-toxic and bio-compatible. Phyto-medicine thus is a real green chemistry where we usually use technologies to understand chemical structure but in reality such technologies are not required for drug formulation. Heterogeneous phyto-antibiotics are best to use as drugs and is a new concept of green chemistry to avoid pollution of complicated organic synthesis.

Figure 1 showed the structures of antibiotics that we used from 1940-2000 to cure bacterial infections. However, beta-lactamases in plasmids inactivated the ampicillin and amoxicillin and such

infections could not be cured by penicillin. Then, the Cephalosporin higher derivative of penicillin were discovered in 1970s. Cefotaxime is used even today but many bacteria acquired blaCTX-M beta-lactamase that could destroy the cefotaxime. Lately, imipenem, meropenem and doripenem like synthetic chemicals introduced as important Carbapenem drugs that also inhibited the bacterial cell wall peptidoglycan biosynthesis. However, such drugs are very toxic and costly and also requires hospitalization and constant doctors supervision. Most painful aspect of the drug development was the fact that blaNDM1 beta lactamase generated in plasmids gave drug resistant to all penicillin, cephalosporins and carbapenems with different degrees [8]. The similar fact was observed for other antibiotics shown in figure 1. In table 1 we have presented the different mdr genes that inactivate antibiotics. Importantly, some membrane bound bacterial enzymes like acrAB and mexAB/CD/EF could remove antibiotics from bacterial cytoplasm rendering drug resistant. In truth, early plasmids were 3-15 kb long but now-a-bay one can isolate 100-500 kb plasmids from *E. coli, P. aeruginosa, S. entirica, S. aureus* and *K. pneumoniae*. We found such drug resistant bacteria could be inhibited by phyto-extracts.

**Figure 1:** Chemical structures of few old antibiotics including new derivatives of penicillin.

| Genes | Full name of subclass | Amino acids | Accession number | Protein Id number | Inactivates antibiotics |
|---|---|---|---|---|---|
| bla | blaTEM | 286 aa | J01749 | AAB59737 | Ampicillin |
| | blaCTX-M | 291 aa | X92506 | ABN09669 | Cefotaxime |
| | blaSHV | 286 aa | X98098 | AAD37412 | Ampicillin |
| | blaKPC | 293 aa | AF297554 | AAG13410 | Amoxicillin |
| | blaNDM1 | 270 aa | KC539430 | AGC54622 | Most penicillins |
| | blaOXA1 | 276 aa | AF227505 | AFG30109 | Oxacillin |
| | blaIMP | 246 aa | EU352796 | AAB30289 | Imipenem |
| | blaVIM | 266 aa | AJ291609 | ABV21756 | Imipenem |
| | blaAMP-C | 382 aa | AF124204 | AAD28044 | Ampicillin |
| | blaSPM | 276 aa | AY341249 | AAR15341 | Ampicillin |
| tet | tetA | 399 aa | X75761 | CAA53389 | Tetracycline |
| | tetA | 424 aa | HM453327 | AHC55487 | Tetracycline |
| | tetB | 401 aa | KP899806 | AKJ20239 | Tetracycline |
| | tetC | 396 aa | KC590080 | AGL61405 | Tetracycline |
| | tetD | 394 aa | AB089602 | BAC67150 | Tetracycline |
| | tetE | 405 aa | JN315882 | AEW70668 | Tetracycline |
| str | strA | 267 aa | M28829 | AAA26443 | Streptomycin |
| | strB | 278 aa | LN555650 | CED95339 | Streptomycin |
| cat | catB3 | 210 aa | EF516991 | ABP52023 | Chloramphenicol |
| aac | aacA1 | 185 aa | AB061794 | BAB72153 | Aminoglycosides |
| | aacA4 | 152 aa | JN596279 | AEZ05102 | Aminoglycosides |
| | aac(3') | 286 aa | M62833 | AAA21890 | Aminoglycosides |
| | aac(2') | 210 aa | U72743 | AAB41701 | Aminoglycosides |

| | | | | | |
|---|---|---|---|---|---|
| aad | aadA1 | 263 aa | AF324464 | AAK13440 | Streptomycin |
| | aadA4 | 263 aa | AY138986 | AAV34365 | Streptomycin |
| | aadA5 | 262 aa | KT175895 | ALO62079 | Aminoglycosies |
| | aadA7 | 265 aa | DQ520937 | ABG01709 | Aminoglycosides |
| aph | aph(6') | 266 aa | X01702 | CAA25854 | Streptomycin |
| | aph(4') | 341 aa | V01499 | CAA24743 | Aminoglycosides |
| | aph(3') | 264 aa | U32991 | AAA85506 | Streptomycin |
| | aph(2') | 294 aa | NC_018107 | WP_000155092 | Erythromycin |
| RND | acrAB/EF | 1027aa | M94249 | WP_001132469 | Acridines |
| | mexAB/CD | 1045aa | U57969 | WP_023101049 | Ethidiumbromide |
| MFS | norA | 388 aa | D90119 | BAA14147 | Ketolides |
| | mdtE | 385 aa | CP000247 | ABG71588 | Acridines |

**Table 1:** Major Classification of Different MDR Genes in bacteria.

The rapid increase of population required large scale food production leading to excessive industrialization, which caused rapid pollution in the environment with depletion of natural resources. Ecological damages occurred due to civilization was first discussed in the Stockholm Conference in Sweden in 1972 where UK stressed the needed laws of Environmental Protection and Scientific Development. Then, great movement appeared in "Brundtland Report" in 1985 and Meetings of Environment Ministers worldwide [9-11]. Further important development occurred at World Summit on Sustainable Development in Johannesburg in 2002, United Nations Conference on Sustainable Development in Rio de Janeiro in 2012 and the Paris Agreement in 2015. Figure 1 showed the green fulfilment of major areas to feed and sustain 7000 millions peoples worldwide without damaging the environment. $CO_2$ emission will be increased 14 Gega tons/year in 2010 to 27 Giga tons/year in 2035 (https://sustainabledevelopment.un.org/content/documents/2843WESS2013.pdf, assessed on 29-06-2020). Thus, economic development was questioned to save this Earth where habitats can use water and air safely. In truth, quality of water and air are 10 times higher toxic label and are very unhealthy for human and animal [12]. Due to increase of metal industry, many heavy metals were found in water and bacteria had acquired metal resistant and metal efflux genes in plasmids where mdr genes already had accumulated due to repeated use of oral antibiotics for long time inhibiting gut bacteria [13]. The length of MDR plasmids in bacteria now have increased from ~5000 bases in 1950s to 100000-500000 bases in 2020. Human Genome Project has not disclosed data on changes of gene sequences with time in human subject which will have catastrophic message. If a bacterial plasmid has three to five mdr genes then why the plasmis sizes increased to 100 kb to 500 kb! Because average size of gene was 2000bp and five genes caused addition of 10 kb only giving maximum size into 15+10 = 25 kb. The answer was that many plasmids acquired metal efflux genes and metalions reduction genes as well as metal chelating genes. As we discussed above that heavy metals were accumulated in water and soil activating bacteria to generate genes involved in metal ions metabolism. Still in five to ten such genes in plasmid could be found increasing the plasmid maximum size to 25+20 = 45 kb. In fact bacterial plasmids had too many retrotransposons, DNA topoisomerases and recombinases as well as integrases, the enzymes that facilitated the new gene creation by cutting and joining of DNA fragments in bacteria. But then such 20 genes could be assigned in 45+40 kb = 95 kb plasmid. Rest increase of sizes were happened due to recombination with F' plasmids (63.5 kb) containing 20-30 Tra and trb genes involved in bacterial conjugation that stabilize the plasmids *in vivo*. Further, to regulate the plasmid transcription of so many genes, five-ten transcription factors were introduced as well as many genes involved extra cellular functions (virulence, glycolytic, bacteriophage genes, plasmid segregation) as required by bacteria in stress [14,15]. Such discussion proved the bacteria changing very much with time and plants are also adapting to save itself from bacteria by producing new ex-metabolites that could kill or retard the bacterial growth. We find few plant extracts are very good source of phyto-antibiotics and we have presented data in support of our view that green antibiotics are safe haven for India where 70% peoples are poor residing in villages.

**Figure 2:** Sustainable Development Programs.

## Research development of green organic synthesis for sustainable development

Mega Industries were first developed in UK and USA and thus strategies for environmental problem-solving non-toxic methods were developed in the developed nations since 1990s. The Environmental Protection Agency (EPA) performed a significant role in developing green chemistry through its pollution prevention programs, funding, and worldwide networks. For example, the 2005 Nobel Prize for Chemistry was awarded, to Yves Chauvin, Robert Grubbs and Richard Schrock, for green synthesis [9]. Rapid de-forestation is a great problem today with increase industry and housing. As a result $CO_2$ concentration considerably has increased in air with ~2°C temperature rise of Earth and melting of ice with increase of water label of sea has also an alarming signal. Use of herbal-drug needs plantation of large area of land and will stabilize the earth's atmosphere.

Sometime twelve principles of green chemistry are which is proposed by John Warner essential to follow [10]: (i) Prevention of waste production (ii) maximize recovery of product/atom economy (AE) (iii) environmental friendly products or no toxic product (iv) high efficiency product like high specific activity of enzyme (v) solvent free reaction using innocuous solvents (vi) energy efficiency (vii) catalytic reusable reagents/renewable raw materials (viii) shorter steps synthesis (ix) use of catalytic amount rather than stoichiometric amount of reagent (x) degradable by products; (xi) real-time monitoring for pollution prevention and (xii) inherently safer chemistry for accident prevention [1]. In truth, many of such problems will be eliminated by green herbal drugs.

Organic synthesis usually creates complex and toxic products and also uses toxic reagents and chemicals. The green synthesis development could be divided into different categories: (i) solvent free catalysis; (ii) Microwave induced green synthesis; (iii) Aqueous-mediated green synthesis; (iv) catalysis green synthesis etc. We will give few examples of such green synthesis which are environment friendly.

## Solvent free catalysis

Organic synthesis is mediated in different organic solvents and those create hazardous by-products. During evolution of green chemistry, novel methods were developed without use of organic solvents (acetone, pyridine, methanol, acetonitrile). Loh., *et al*. (2000) disclosed an efficient and environmentally friendly method for Mukaiyama aldol reaction of ketene silyl acetyls with different aldehydes adding trace of DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) under solvent free conditions at room temperature [14].

Recently, Kumar and Maurya disclosed an organo-catalysed reaction of an aldehyde, ester, cyclic diketone and ammonium acetate to prepare hydroxyquinoline derivatives [16]. A method for stereo-selective synthesis of 1,3-amino alcohols in presence of indium trichloride catalyst known as Mukaiyama aldol reaction of keto ester under solvent-free conditions [17]. Thus, they used the organo-catalysts for reaction of aceto-acetate ester, cinnamaldehyde and anilines to prepare N-aryl 5-unsubstituted or 5,6-unsubstituted 1,4-dihydropyridines. Kumar., *et al*. also described Zirconium chloride as efficient catalyst which catalyzed aldol reaction of appropriately substituted aromatic aldehydes with aromatic ketones to prepare 1,3-diaryl-2-propenones without the formation derivatives under solvent free conditions. The reaction is clean and environmental friendly for the synthesis of variety of 1,3-diaryl-2-propenones. Ranu., *et al*. collectively reviewed different methods for the production of quinolines and dihydroquinolines by a simple one-pot reaction of anilines with alkyl vinyl ketones on the surface of silica gel impregnated with indium (III) chloride under microwave irradiation without any solvent [18]. Majhi., *et al*. also has disclosed a method for oxidative arylation of vinyl arenes by aryl radicals generated in situ from arene diazonium fluoroborates in presence of ascorbic acid at room temperature and in the absence of any light irradiation. A series of diversely substituted 2-aryl acetophenones have been obtained in good yields by this procedure [19].

## Microwave induced green synthesis

Microwaves (0.3 GHz-30 GHz) is non-ionizing radiations with higher wave lengths which stays between radio wave (RF) and infrared (IR) frequencies and now a day is used in cooking. In microwave reactions selective homogeneous absorption of electromagnetic waves produced affecting polar molecules where as non-polar molecules being non-reactive to microwaves. In microwave induced organic reactions both solid and liquid medium could be utilized for chemical reaction. The ability of a specific solvent to convert microwave energy into heat at a given frequency and temperature is determined by the so-called loss tangent (tan δ). A reaction medium with a high tan δ at the standard operating frequency of a microwave synthesis reactor (2.45 GHz) is required for good absorption and efficient heating where tan $\delta = \varepsilon''/\varepsilon'$ and $\varepsilon'$ is the dielectric constant. Microwave ovens offer a clean and cheaper alternative to oil baths for many organic reactions. Haque., *et al*. have synthesized environmentally friendly three chitosan Schiff bases under microwave irradiation by the reaction of chitosan and aldehydes and 4-hydroxy-3-methoxybenzaldehyde [19]. Bogdal disclosed a method of synthesis of aromatic ethers by reaction of phenols with primary alkyl halides under microwave heating. Similarly, Li., *et al*. reported the microwave-assisted coupling of phenols as Reviewed in Bandyopadhyay., *et al*. 2017 and others [20-24]. Promising results were demonstrated for the Bischler-Napieralski reaction, the WolffKishner reduction, free radical dehalogenation reactions, and other standard synthetic operations [25].

## Aquous-mediated green synthesis

Water is a very abundant, non-toxic and low cost entity in nature. It has relatively high dielectric constant and density compared to organic solvents. Aqueous mediated reactions are useful and more environmentally friendly alternatives to their toxic organic solvents. A water-mediated method for the preparation of pharmaceutically important and diversely functionalized different heteroaryl-dione compounds has been developed using one-pot [26]. A simple, efficient and green procedure for the synthesis of 3-carboxycoumarins has been described using 2-hydroxybenzaldehydes and Meldrum's acid in aqueous moist conditions at room temperature [27]. WU., *et al*. have devised a Barbier allylation reaction mediated by many metals like Mn, Zn and indium and others in aqueous medium [28].

## Ancient Green Medicine Approaches use only plant products

Charaka Samhita, Sashruta Samhita and Atharva Veda are >5000 years old Sanskrit books describing Indian ancient medicine using phyto-extract to cure diseases. The Chinese book (2500 BC) on medicinal roots and grasses "Pen T'Sao," described by Emperor Shen Nung with 365 drugs preparation methods using phyto-extractslike cinnamon, camphor, podophyllum, ginseng and ephedra. The holy Bible, the Jewish book the Talmud, Homer's epics The Iliad and The Odysseys (800 BC) had described ancient methods of herbal medicine. Dioscorides' medicinal book described many useful medicinal plants like willow, onion, camomile, garlic, nettle, marsh mallow, ivy, parsley, sage, common centaury, coriander, and false hellebore. Pliny the Elder (23-79 AD) also described thousand medicinal plants in the book Historia Naturalis. Liber Magnae Collection is "Simplicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197-1248) also has depicted many useful plants as medicine. Marco Polo (1254-1324) visited Asia, and America and Vasco De Gama's journey to India (1498), resulted in the development of many medicinal plants being brought into Europe for drug research. Between 16th and 18th centuries medicinal plant extracts used in concentrated forms with animal parts as well as minerals. The major concepts were if you concentrate the drug parts, then it cures the disease effectively. However, toxicities of Opium, Styrax, Colchicum and Ricinus were also reported. Herbal pharmacopoeias PhEur 6, USP XXXI, and BP 2007 prescribed plant drugs having good medicinal value. Present day purification by Thin Layer Chromatography, Gel Filtration using Sephadex-G columns and HPLC gave pure chemicals to cure diseases and chemical nature of the active compounds were determined by MASS, NMR and FTIR spectrometry [29,30].

## Research Progress in Green Antibiotics to cure cancer and malaria

Use of Green antibiotics is new approach combining sophisticated modern techniques and ancient methods to discover herbal-drugs against pathogens. Well known green antibiotics are: quinine and artemisinin against malaria disease; Taxol and etoposide against cancer and reserpine against bacteria [29]. The plant chemicals were first solubilised in water or ethanol or mixture of both and then silica-gel column chromatography was performed to separate abundant chemicals. Then, after assaying the biological potency, such partially pure chemicals were further purified by

partition chromatography between different organic solvents (methanol, acetic acid and acetone etc.) using High Performance Liquid Chromatography (HPLC). Quinine, tenoposide, artemisinin, taxol, resveratrol, serpentine and many other new compounds are plant-derived >90% pure antibiotics eradicating malaria, cancer and bacterial infections in recent times [31-33].

Taxol was purified from the bark of *Taxus brevifolia* or *Taxus wallichiana* and very effective drug for many cancers. Raw material was grounded and then extracted in methanol (1:3) solvent in a Soxhlet apparatus. The extract mixed with silica gel and purified and then dried in rotary evaporator under vacuum at $40^0$C. $CO_2$ supercritical fluid extraction (LAB SFE from Separex) at 500 bar and $50^0$C for 3 hrs is a very good extraction method (231). The reported fact was that 1200 Kg bark produced 28 Kg crude taxol from which 10g pure taxol was obtained. Such pure drug when injected i.v. was shown to kill tumour effectively. In truth, bark from 360000 trees were used per year in the United States for cancer therapy. Ethanol extract of *Camptotheca acuminate* bark was effective to inhibit cancer cell lines and xeno-graft in mice. Camptothecin was purified and was shown to inhibit DNA replication by inhibiting DNA topoisomerase I but not DNA topoisomerase II modulating DNA replication and transcription.

Quinine was isolated in 1820 from the bark of a cinchona tree. Nicolás Monardes (1571) and Juan Fragoso (1572) first described the cinchona tree bark extract to cure diarrhoea But Agostino Salumbrino (1602) likely used bark extract first to reduce the fever of malaria. The name was derived from the original Quechua (Inca) word for the cinchona tree bark, quina or quina-quina, which means "holy bark". Prior to 1820, the dried bark was grounded and then mixed with wine and the extract was then drunk. Dutch explorer managed to grow the plants in their Indonesian plantations in 1913 onwards with production of 22 million pounds of cinchona. Quinine remained the main anti-malarial drug until after World War II, when cheap synthetic drug chloroquine largely replaced it [32]. However, drug resistant to chloroquine was now prominent and still quinine was prescribed.

Artemisinin (Qinghaosu) was isolated from *Artemisia annua* leaves (wormwood) in 1972 as anti-malarial drug against chloroquine-resistant *Plasmodium falciparum* malaria by Chinese scientist Tu Youyou. It was extracted with ethanol, hexane and petroleum ether but liquid $CO_2$ supercritical extraction was used in commercial isolation now-a-day. In a another method, silica gel chromatography in acetonitrile medium following slow water addition creates pure crystal of artemisinin with good recovery [33]. Recently, Sanofi Pharmaceutical has started production of artemisinic acid and yeast cells engineered with CYP71A gene in expression plasmid used to produced artimisinic acid which was then converted to artemisinin by chemical synthesis.

Herbal drug production maximizes the production of raw plant material in unfertilized lands and some tree parts are utilized as active ingredients isolation and the rest is used as wood products and cooking energy. The land was replanted annually or roots and barks could be collected 2-3 times time in a year for sustainable time. The land became fertile for vegetable and fruits cultivation within few year and whole process is safe, environment friendly. The extraction solvent like ethanol, methanol, acetone and ethyl acetate could be recycled avoiding the production of waste [8].

Thus, there are huge potential of plant-derived antibiotics to cure bacterial infection as well as for the treatment of diabetes, malaria and cancer. We have isolated CU1 (a saponin antibiotic) from Cassia *fistula* bark having RNA polymerase target where as NU2 flavones has DNA topoisomerase I target isolated from *Suregada multiflora* root [34-37]. Further, we have started characterizing big Sal tree (*Shorea robusta*) inner bark extract and very small Verenda tree (*Jatropha gossypiifolia*) root extract as important anti-bacterial and those chemicals were purified by TLC method (unpublished). We will present the modern scientific development in support of green phyto-drug against multi-drug resistant bacteria that we are doing in our laboratory at OIST in collaboration with many National Laboratories.

## Materials and Methods

### Preparation of organic phyto-extract (MDR-Cure) and phytochemical purification

We have collected the plants parts from the Midnapore district of West Bengal. The barks of *Suregada multiflora* (local name: Ban-Narenga), *Cassia fistula* (local name: Bandhorlathi) were collected on July 2019 from medium sized tree at Midnapore district of West Bengal, India [30]. *Syzygium aromaticum* – flower buds (labanga spice) and *Cinnamomum zeynalium*-Bark (darchini) were purchased from grocery stores at Midnapore city. Each 10gms

semi-dried plant or spice parts were suspended in 40 ml ethanol or ethanol-ethylacetate (1:1) for overnight. Then concentrated 5-10 times at room temperature and 50 µl used for Kirby-Bauer agar hole assay. We termed MDR-Cure lotion which was a mixture of five plants extracts and cured MDR nail infections in human (unpublished). The crude plant extract was purified by Thin Layer Chromatography (TLC) using Methanol, water and Acetic acid as mobile phase (50:40;10) for 0.8hr-1.2 hrs. Organic molecules were seen and recovered by UV shadowing and was eluted in ethanol from silica-gel, centrifuged at 10000rpm and dried at room temperature. In case of large preparation, 1000 ml methanol and 200g plant parts was used and about 32 TLC plates (15x20 cm) used to get 100 mg >95% pure chemical as judged by HPLC [7,35].

## Isolation of multi-drug resistant bacteria

Usually, we plated 100 µl Ganga River water in LB+Agar+50 µg/ml Ampicillin plate to get individual drug resistant colonies during over night incubation at 37°C. For cefotaxime 25 µg/ml drug and 200 µl water was used. To get meropenem resistant bacteria, we used 10 ml water and 2 ml 6xLB media and meropenem at 5 µg/ml was added. After overnight incubation, 1 µl drug resistant bacterial suspension in 100 µl LB media was plated on LB+Agar+10 µg/ml meropenem and single colony drug resistant bacteria was grown in LB+20 µg/ml meropenem to get high meropenem drug resistant bacteria. Then, such drug resistant bacteria was used drug sensitivity test using many antibiotic filter papers obtained from HiMedia and genomic DNA was isolated and sent for 16S rRNA gene sequencing by SciGenom Limited, Kerala (30).

## Isolation of bacterial plasmid DNA, PCR and sequencing

The plasmid DNA was isolated from over night culture using Alkaline-Lysis Method. Simply, to bacterial pellet 100 solution I was added and vortexed. Then 200 µl of cold Solution II added to make transparent solution and then 150 µl cold of Solution III was added and mixed well for precipitation. After 10 min the solution containing huge white precipitate of chromosomal DNA-cell debries were removed by centrifugation at 10000rpm for 10min.. To clear solution then added 1 ml 99% ethanol and centrifuged at 10000 rpm for 10 min at 4°C. Plasmid DNAs from four such preparation were combined and the tRNAs were removed by Rnase A treatment with DNase free RNase, extracted with phenol-chloroform and ethanol precipitated again and dried at room temperature. Finally plasmid DNA was dissolved in 50 µl TE buffer and was stored at -20°C until use. 0.8% agarose gel lecetrophoresis in 1x TAE buffer at 50V for 4 hrs was performed to see the plasmid DNAs after staining in 0.5 µg/ml ethidium bromide and UV illumination (30). 16S rDNA gene colour Sanger's di-deoxy sequencing was performed by SciGenom Limited, Kerala, India). PCR amplification was performed using 1 unit Taq DNA polymerase, 0.25 mM dXTPs, 1.5mM $MgCl_2$, 20ng DNA template for 35 cycles at 95°C/30" (denaturation)-52°C/50"(annealing)-72°C/1.5' (synthesis). The product ran on a 1% agarose gel in 1X TAE buffer at 50V for 2 hrs and visualized under UV light and photograph was taken. The primers for 16S rRNA amplification and mdr genes are given below. NCBI BLAST analysis was performed for bacterial specific gene analysis (www.ncbi.nlm.nih.gov/blast) and data was submitted to GenBank [7].

## Assay of Escherichia coli RNA Polymerase using 3H-UTP

*E. coli* RNA polymerase assay was performed as described by Lowe., *et al*. (1979) with minor modification [34,35]. The reaction (Rx 40 µl) was performed with transcription buffer (40 mM Tris-HCl pH 7.9, 200 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 14 mM β-ME, 200nM each ATP, GTP and CTP. 50µM UTP, 2µCi $^3$H-UTP (BRIT, Hyderabad, India), 1.5 µg calf thymus DNA and 1U RNA polymerase at 37°C for 20 min. The reaction mixture was spotted onto DEAE-paper pre-socked with 5mM EDTA. The filter paper was then washed with 5% di-sodium hydrogen phosphate, thrice with water and finally with 95% ethanol and dried. The filters were placed into 10 ml toluene-based scintillation fluid and counts were recorded on a Tri-CARB 2900TR scintillation counter. Data was normalized and plotted. Rifampicin (10 mg/ml in basic ethanol) was used as standard drug and phyto-chemicals (4 times TLC purified; ~10 mg/ml in DMSO) were used.

© Method of chemical characterization of active phytochemicals

## Mass spectra procedure

A mass spectrum is intensity vs. m/z (mass-to-charge ratio) plot (histogram) which is unique for any plant alkaloid or other chemicals. Usually, pure chemical is hit by laser and +ve charged particles are detected in high intensity magnet, separating molecular ion and its fragments using a Mass Spectrometer. Lighter ions get deflected by the magnetic force more than heavier ions based on Newton's second law of motion, F = ma, J. J. Thomson, Francis Aston, Arthur Dempster, and Kenneth Bainbridge discovered and developed mass spectrometer. Matrix-assisted

laser desorption/ionization (MALDI) spectroscopy computer algorithm was assembled in a way you may know the structure of your unknown sample immediately from computer database [38]. However, such software is costly and may not be available to you.

### NMR-spectrosopy procedures

Nuclear Magnetic Resonance (NMR) is a spectroscopic technique to detect local magnetic moment around odd atomic nuclei bombed with radio waves with a wavelength 75-0.5m. The most commonly used small molecules are hydrogen ($^{1}$H) and carbon ($^{13}$C) but other $^{11}$B, $^{19}$F, $^{23}$Na, $^{31}$P,$^{35}$ Cl etc also have been used using NMR [35]. For data analysis, NMR absorption spectra was adjusted to Chemical Shift (δ) using Tetra Methyl Silane (TMS) as standard and data was expressed as ppm (parts per million). TMS is chemically inert, magnetically isotropic, miscible with most organic solvent and absorbs at higher frequency than all common types of organic protons [39].

### Infra-Red spectroscopy procedure

Infrared spectroscopy gives an idea of functional groups of a compound. Wave number (v $cm^{-1}$) is used to measure the infrared absorption at 4000-667 $cm^{-1}$ (2.5-15 μ Wavelength). A nonlinear molecule with n atoms has 3n-6 vibrational modes of stretching, rocking, scissoring, wagging and twisting giving a idea of functional groups of the molecule. Bending vibration occurs at lower wave number than stretching vibrations. Carbon-carbon triple bond absorption at 2300-2000 $cm^{-1}$, double bond absorption at 1900-1500 $cm^{-1}$ and single bond at 1300-800 $cm^{-1}$. O-H stretching absorption at 3570-3000 $cm^{-1}$, C-H stretching at 3030-2860 $cm^{-1}$ and same bending at ~1460 $cm^{-1}$ were reported to characterize polyphenols. C = O stretching at ~1725 $cm^{-1}$ and-N-H stretching at 43400 $cm^{-1}$ and same bending at ~1650 $cm^{-1}$. -C-N stretching absorption at 1350 $cm^{-1}$ but C = N at ~2200 $cm^{-1}$ [36]. Typically, 5 mg purified dry active chemical was mixed with 200 mg IR-grade KBr and the tablet was prepared at 13 mm. Die SET at 10 fKg/$cm^2$. Spectra were taken with a Perkin Elmer Spectrum 100 FT-IR Spectrometer for 10 min [40].

## Result

### Isolation and characterization of multidrug resistant bacteria from Ganga River water, Milk and chicken meat

Water from Ganga River was collected in the morning at Babughat (Kolkata-700001). Milk was g/ml collected directly into 15 ml graduated plastic tube from Midnapore Khatal opposite Midnapore station. Industry milk and card were tested but had no bacteria. Chicken meat was collected from meat shop at local bazaar and immediately 1gm meat pulverized with 10 ml LB media and centrifuged at 1000 rpm for 5 min in a doctor's table top centrifuge. MDR bacteria were selected in LB-Agar plus different drugs plates. For that 100 μl water or milk or meat extract was spread onto 10 cm LB-agar plate containing 50 μg/ml of each ampicillin, ciprofloxacin, azithromycin and 20 μg/ml tetracycline.

Drug resistant individual colony was taken and was grown in LB media plus drug. Plasmid as well as chromosomal DNA were isolated. 16S rRNA sequencing performed and the sequence was BLAST search to see perfect homology. Further, plasmid DNA used to perform PCR using mdr genes specific primers. Then drug sensitivity tests were perform using different antibiotics paper disks to confirm MDR phenotype.

A proto type picture of drug sensitivity test was shown in figure 3A where a *Escherichia coli* KT-1_mdr and *Pseudomonas aeruginosa* DB-1_mdr bacteria were used [7,30]. Figure 3B showed the plasmid isolated from chicken meat derived three bacteria resistant to streptomycin, ciprofloxacin, and azithromycin (panel-A) and also *E. coli* KT-1_mdr isolated from Ganga river water. It confirmed that *E. coli* KT-1 bacteria had so many plasmids that gave a smear. On the other hand chicken derived MDR bacteria gave distinct plasmid bands about 30 kb as well as much larger plasmids (>100 kb) that were stayed near the lane due to very slow mobility in 1% agarose gel. Figure 3C showed the PCR analysis of *Escherichia coli* KT-1_mdr plasmid DNA using mdr genes (bla, tet, amp, acrA) specific primers as originally described in Chakraborty, 2015 [30]. It showed the tetracycline resistant gene (tetC gene) highly amplified as also acrA drug efflux gene. Figure 3D showed the DNA sequencing data 16S rRNA gene of *E. coli* KT-1 as well as *P. aeruginosa* DB-1 obtained from chromosomal DNA. It was concluded as *E. coli* and *P. aeruginosa* by BLAST search and also confirmed the sequence variation between the 16S rRNA gene of *E. coli* and *P. aeruginosa*. We have studied in detail of CU1 phytochemical from *Cassia fistula* bark. We had shown that CU1 inhibited *E. coli* RNA polymerase very specifically as compared to rifampicin (Table 2).

**Figure 3A:** Antibiotic paper disk assay of few superbugs (7). Those are KG-12 and KT-1 strains. In (A) KG-12 isolate: 1= MET-10 (methicillin), 2 = CAZ-30 (ceftazidime), 3 = AT-50, 4 = COT-25 (cotrimoxazole), 5 = LOM-15 (lomofloxacin), 6 = VA-10 (vancomycin), 7 = AK-10 (amikacin), 8 = LZ-10 (linezolid), 9 = TGC-10 (tygecycline) and 10 = IMP-10 (imipenem). In (B) KT-1 isolate: 1 = VA-10, 2 = AK-10, 3 = LN-10, 4 = MET-10, 5 = CAZ-30, 6 = AT-50, 7 = COT-25, 8 = LOM-15, 9 = TGC-15, 1ane 10 = IMP-10.

**Figure 3B:** Isolation of plasmids from MDR bacteria: (A) bacteria isolated from chicken meat with selection of streptomycin, ciprofloxacin and azithromycin. (B) Ganga river water MDR E. coli KT-1. L/H means Lamda virus DNA digested with Hind III restriction endonuclease. pBR means ccc-pBR322 plasmid DNA. The >100 kb plasmids were found near the lanes. *E. coli* KT-1 contains too many plasmids giving a smear.

**Figure 3C:** PCR amplification of mdr genes from plasmid DNA isolated from MDR E. coli KT-1. Tet gene highly amplified and to lesser extent mcr and acrA drug efflux gene. 16S rRNA gene amplification used for sequencing is also shown. DNA marker is 100bp ladder.

**Figure 3D:** 16S rRNA gene di-deoxy sequencing from isolated MDR E. coli and P. aeruginosa. The difference positions were labelled by star marks. Reverse primer mediated sequencing pattern (~140bp) was shown here comprising 1197-1057 (reverse strand) rRNA gene of *E. coli* (accession no, AE014075, region nt. 235000-237000).

## Different plants parts and Purification of phytochemicals from ethanol extracts on preparative silica gel thin layer chromatography

Figure 4A gave the identity pictures of labanga and derchini, two important spices of India. Figure 4B gave the identity picture of Bandor-lathi (golden showers), Sal and Verenda. We presented the TLC profile of Derchini extract in figure 4C and DU1 as well as DU2 phyto-chemical were both active in Kirby-Bauer assay to inhibit bacterial growth presented in figure 4D. Cloves and Derchini oils were reported having beta-caryophyllene (12%), methyl salicylate (8%), phenylpropanoids (kaempferol, thymol, euginol), cinnamaldehyde), and triterpenoids (oleanolic acid, stigmasterol). Cloves or labanga extract on TLC did not produce a good band of phytochemicals and all fractions appeared to be some activity (Figure 4E). On the contrary, crude extract was excellent to inhibit MDR bacteria and well as E. coli DH5α, non-mdr strain (data not shown). We also presented some preliminary data of ethanol extract of fruits peel extract of panifal (Figure 4F). It was found that fruits peel ethanol extract of *Trapa bispinota* had a great antibacterial activity (lane 4) but major inner mass (white) of fruit had no antibacterial activity. However, Cassia fistula bark extracts had some activity even in bark water extract (lane 5 and lane 6). The *T. bispinota* plant grows in water and the fruits were immerged in water. Thus, we thought that fruits peels might have some antibacterial properties. A typical NU3 fraction from Suregada multiflora root extract HPLC profile was presented in figure 5. The retention time of such aminoglycoside was high (10min). Note that some terpenetine bromophenol phyto-drug from *Cassia fistula* bark has less retention time (3 min) (Data not shown).

**Figure 4A:** Photographs showing (a) Labanga (*Syzygium aromatium* flower buds) and (b) Derchini (*Cinnamomum zeynalicum* inner bark) and both ethanol extract had excellent antibacterial activities.

**Figure 4B:** Important Midnapore district plants that were used here for isolation of phytochemicals to inhibit the growth of MDR bacteria (A) Bandorlathi (medium size tree), (B) Sal (large tree) and (C) Varenda (very small tree).

**Figure 4C:** TLC purification of DU1 and DU2 phyto-chemicals from Derchini ethanol extract. I = TLC plate, II = Load of crude extract, III = TLC done; IV = UV shadow of III and V = repeated TLC of DU2 obtained from IV.

**Figure 4D:** Antibacterial assay of Derchini phyto-chemicals using *E. coli* KT-1_mdr. TLC-purified DU1 chemicals has colour but DU2 is colourless.

**Figure 4E:** Preparative TLC of ethanol extract of Labanga. All fractions (LU1, LU2, LU3) had some antibacterial activities but high fluorescence (Lane) have no activity).

**Figure 4F:** Inhibition of *E. coli* KT-1 bacteria by *Trapa bispinota* fruits as compared to *Cassia fistula* bark ethanol and water extracts

**Figure 5:** HPLC purification of phyto-chemicals from TLC-purified ethanol extract of *Suregada multiflora* root. The aminoglycoside type chemical eluted at 10 min.

| | | |
|---|---|---|
| Complete | 1325 cpm | 100% |
| Complete + Rifampicin | 63 cpm | 4.75% |
| Complete + CU1 | 75 cpm | 5.66% |
| Complete + CU3 | 1296 cpm | 97.8% |
| Complete + RNase | 87 cpm | 6.5% |
| Complete + DNase | 1123 cpm | 84.75% |

**Table 2:** Inhibition of *E. coli* RNA polymerase byCU1 phyto-chemical of *Cassia fistula* bark ($^{3}$H-UTP incorporated in RNA and Percent activity).

## Modern technologies to understand the chemical nature of active phyto-chemical

Modern Day drug development is costly and needs many sophisticated high priced instruments to understand the chemical structure and mode of action. Herbal drugs do not need such technologies but in modern days without such characterization nobody will believe your drug. Thus, we believe on such study but our MDR-Cure drug is a combination of five different ethanol extracts. However, we may use low cost TLC method to purify abundant phyto-chemical that has good antibacterial activity on MDR isolates. Further, any drug must be passed the rules and regulations of clinical studies involving four stages in human and usually requires few years. But in case of herbal drugs, such studies might be relaxed if ancient long term use in human was recorded in ayurvedic books. But local traditional medicine may not be recorded in ancient books and we have to look Pubmed (NCBI) for any record. Moreover, more you know about the drug, then toxicity profile and dose determination and chemical composition of the drug will be performed. We usually go to national laboratories for such studies and needs minor payments as service charges [7,37].

We presented atypical mass spectra of a TLC-purified phyto-chemical from *Suregada multiflora* bark. It showed the low intensity molecular mass at 455.23 and major sub-fragments are 402, 353 and 312 (Figure 6) and time course plot (data not shown) showed different fragments indicating the chemical was not pure.

A proton NMR spectra of a plant derived chemical LU2 (Derchini) was presented in figure 7. We can get the different atoms linked to hydrogen from standard chart of delta. $\delta$ = 3.64 ppm (weak) for R-OH, 2.09 ppm (medium) $CH_3$-C = O, 1.56 ppm (very strong) $CH_3$-C-X, 1.28 ppm (strong) for C-C-H and 0.99 ppm (broad medium) for $R_2NH$ were obtained. $C^{13}$ NMR and 2-D NMR spectroscopy techniques can detect the bond between individual atom in a molecule but in India such experts are limited.

A represented FT-IR spectra of *Suregada multiflora* NU3 phyto-chemical was presented in figure 8 indicating -$NH_2$, -OH, -$CH_3$ functional group for a aminoglycoside antibiotic [7]. We found many functional groups and likely the compound is a glycoside. Peak at 3426.9 is for -N-H stretching and -O-H stretching; 2960.1, and 2849.8 $cm^{-1}$ are for -$CH_3$ stretching; 1631.8 and 1536.1 $cm^{-1}$ are for CO-$NH_2$ scissoring; 1462.9, and 1387.9 $cm^{-1}$ represent -OH bending likely phenolics; 1259.1 and 1230.6 $cm^{-1}$ represent C-$NH_2$; 1134.0 $cm^{-1}$ for C-C-C bending; 802.4 $cm^{-1}$ for -$NH_2$ wagging; 719.8 $cm^{-1}$ may represents -$CH_2$- rocking.

Two years laboratory closer during COVID-19 pandemic drastically hindered the progress of work and more work to be perform. But clearly we have isolated the MDR bacteria and used such bacteria for our phytochemical analysis from *Suregada multiflora* (Naringa), *Cassia fistula* (Bandotlathi), *Shorea robusta* (Sal), *Jatropha gossipifolia* (Varenda), *Trapa bispinota* (Panifal) and two spices derchini *(Cinnamomum zeynalicum)* and labanga (*Syzygium aromatium*). However more work needed to get the structures of the active compounds.

**Figure 6:** A typical mass spectra of a TLC-purified NU3 phyto-chemical from *Suregada multiflora* bark.

**Figure 7:** Proton-NMR of LU2 phyto-chemical from Labanga (Clove) in CDCl3. δ = 7.2 ppm indicated benzene nucleus and might be also for CDCl3.

**Figure 8:** Detection of function groups of the active compound NU3 of *Suregada multiflora* by FT-IR.

**Figure 9:** Medicinal plant and herbs garden in Rastrapati Bhaban Estate, New Delhi.

## Discussion

Most of the phyto-chemicals like saponins, phenolics, flavonoids, triterpenes and alkaloids were reported to have inhibitory effect on cancer cells and bacteria as well as fungus pathogens. High content of phenolics and flavonoids in medicinal plants with antioxidant properties were effective in the prevention of age-related diseases. However, such phytochemicals are very labile to heat and light. So during purification process very much care must be take. In our method, plant parts were chopped with knife and overnight extracted in ethanol at room temperature. Dried phytochemicals stored at 4°C. Traditional updated methods such as Maceration and Soxhlet extraction are commonly used at the small research setting or at Small Manufacturing Enterprise (SME) level. Few other modern extraction methods are; microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE), to increase yield at lower cost and may also increase stability of active alkaloids and aromatic compounds [44]. Usually fresh or air-dried sample with grinded plant parts used for extraction by maceration, infusion, percolation and decoction. Maceration means soaking grinded plant part

in a container with a solvent in tight condition and allowed to stand at room temperature for a period of 2-3 days with frequent agitation. For heat stable alkaloid compounds, boiled water could be used for 2 hours for extraction. Accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) are updated methods for quick extraction of bioactive compounds within 2-3 hours [7,25]. But we never used boiling method or Soxhlet extraction method.

We have described that phyto-chemicals have enough power to cure diseases like bacterial infections. During phyto-drug discovery few things to remind: make different extracts in many solvents, concentrate them to get >15 mm lysis zone in Kirby-Bauer agar-hole assay and then perform preparative TLC to check if a specific TLC-band represents high glycoside/flavonoid/terpenetine content with anti-bacterial activity [7]. Many workers have reported other biological activities like anti-diabetic, anti-fungal and anti-cancer activities but such data would not help for commercial production due to low potency and low percentage content [5]. Typically, we suggest, phytochemical must be near <0.05% of dry mass of plant part and >20% of extracted crude extract dry mass. For micro assay, we add 40 ml ethanol to 4g plant part (grinded or chopped 5 mm) and concentrated to 2 ml where 50 µl sample must give >15 mm lysis zone as compared to 20-35 mm lysis zone of standard drug (20 µl 34 mg/ml chloramphenicol or 20 µl of 1 mg/ml meropenem or 20 µl of 50 mg/ml streptomycin). We made standard MDR-Cure Lotion with five anti-bacterial extracts with 50% ethanol extracts of neem tree bark and haldi rhizome (unpublished data). Such extracts killed 50 different MDR bacteria from Ganga river water as well as cow milk, chicken meat, human hair and human blood sample-derived MDR bacteria [30]. Further, we checked that human drug resistant chronic nail infections were cured in five cases (unpublished data). AKC planted a tree of *Suregada multiflora* in 4$^{th}$ floor roof of flat at South Kolkata and within 5 year I collected twice in a year 20g root enough to cure MDR infection in human (unpublished data). Practically in India most people take non-prescription antibiotics in case of fever, diarrhoea and skin infections [37-41]. According to WHO, MDR infections will be prominent in the future and WHO guidelines has warned the MDR bacterial calamity of water resources [5,7]. We are very sure about potency of MDR-Cure and also about the potency of naringa, bandorlathi, varenda, sal trees bark and roots as strong anti-bacterials and much stronger than neem, arjun and haldi. *Cinnamomum zeylanicum* (Derchini) bark extract has potential anti-bacterial and we have purified to some extent (Figure 4D and figure 4F) [44]. *Suregada multiflora* and *Cassia fistula* bark and root ethanol extracts have potential antibacterial compounds. However, natural resources of such plants are diminishing and in truth 100 acres of land plantation is necessary to overcome future catastrophic chronic deadly infections of pan drug resistant bacteria. Alarming fact, MDR-TB and MDR-Typhoid are increasing in India [42-44]. Medicinal plant preservation and plantation must be augmented and green antibiotics or herbal antibiotics are a new challenging field of medicine for the progression of mankind of this Earth. Green technology drug formulations have tremendous use modern days and such technology has welcome by G-20 leaders and WHO. Many scientists are working with green chemistry in this field using nanotechnology and gene therapy [45-54]. We are also discovered few drug targets of phyto-chemicals [52-56]. We argue that scientific development must be accepted but our MDR-Cure drug formulation is very green and in house technology. UN Summits have formulated many action plans but still hunger and ill-health are a problem of society. India has ~130 cores peoples and mostly lower middle class where green antibiotics (MDR-Cure extract) may be a solution to cure rapidly accelerating MDR bacterial infections. Recently, an US survey indicated that MDR infections were co-morbidity in many cases (cancer and Coronavirus infection) where costly 5$^{th}$ generation invasive antibiotics were necessary compounding the cost of the treatment of Corona virus infected patients. During HIV retrovirus infection, more than ten bacteria-fungus infections caused AIDS where no treatment available today.

The Government of India has made many herbal gardens and forests. AYUSH Wellness Clinic has treatment facilities in the stream of Ayurveda as well as Yoga, Unani and Sidda according to ancient Hindu Civilization. National Medicinal Board has described to save medicinal plants like Chandan, Neem, Tejpata, Kapoor, Datura, Dalchini, Jamm, Bel, Hara, Baheda, Amla and more. Yelagiri Herbal Garden of Tamil Nadu, AVS Herbal Garden of Kerala are very famous. Dhanvantri CCMB Herbal Garden of Hyderabad is also notable and LRP Ayurveda Herbal Garden of Maharastra and Kerala Indian Spices and Herbal Garden at Adimali as well. The total forest area in India is 712249 Sq KM (71 millions hectres) which is 21.67% cover of the total area of India. But in West Bengal total area of forest is only 13% (11879 Sq KM) or less of

the State area. A huge montane forest is situated in the Northern West Bengal districts of Alipur Duar, Derjeeling and Kalimpong. Further, you may find some medicinal plants in the mangrove forest of Sundarbans located in Southern West Bengal. My research was conducted with available plants in West Midnapore district of West Bengal. We found Bandorlathi (Golden showers; *Cassia fistula*), Narenga (*Suregada multiflora*), Sal (Dhuno tree; *Shorea robusta*); Varenda (*Jatropha gossypifolia*) as valuable medicinal values particularly to inhibit multi-drug resistant bacteria [49-57]. Biotechnology Department of Ramkrishna Mission at Nimpith (Jaynagar rail station; South-24 Paraganas) has a well established plant tissue culture and micropropagation facility and you may find many medicinal plants. A picture of medicinal plants in the garden of Rastrapati Bhawan Estate was given in figure 9. However, West Bengal needs one 100 acres of medicinal plants conservation and cultivation facility and State as well as Central Government must act urgently to establish such program. The old ponds of villages have jungles where you may find a collection of medicinal plants planted by your grandfather. Sadly, such jungles are rapidly vanishing due to housing. IAKC found *Suregada multiflora* (Narenga tree) at the bank of the Ghosh Pukur (pond at Ekbalpur village, Ghatal subdivision, West Bengal) planted by my grandfather because the leaves of the tree can reduce malaria fever. I found *in vitro* anti-malarial activity in leaves extract (unpublished). *Cassia fistula* was obtained in our campus garden at Midnapore and also could be seen in campuses of IIT-Kharagpur and Jawarlal Nehru University campus, New Delhi. *Jatropha gossypifolia* was found in the railway roads but drastically their population are diminishing [5,7]. We have to preserve our medicinal plants and private sectors plantation programs may be needed. Dabur India Limited is only best company which developed many herbal remedies and Emami Limited has invested in ayurvedic drugs recently Alarming areas of research are immune-disorder, diabetes, fertility control, cancer, AIDS, superbugs and hypertension where medicinal herbs and plants may be greatly beneficial.

Sanskrit books are not familiar to read by Indian students and recently few valuable English books on medicinal plants were published. These books are available in India. All Indian must read those books to know medicinal plants of India and their uses as medicine. I am sure common men and women can cultivate such plants in garden or clay pot in roof top and can prepare ethanol phyto-extracts to cure many diseases. Hence, some information on the books was given below for readers.

Recent Progress in Medicinal Plants: Chemistry and Medicinal Value, Vol 25 - 2009 by V. K. Singh and J. N. Govil. ISBN-10: 1933699159; ISBN-13: 978-1933699158. Publisher: Studium Press (India) Pvt. Ltd. Medicinal Plant in India: Importance and Cultivation Vol 1 - 2019 by S N Ghosh. ISBN-10: 938866809X; ISBN-13: 978-9388668095; Publisher: Narendra Publishing House. Pharmacognosy and Phytochemistry: A Companion Handbook-2019 by Sharada L Deore. ISBN-10: 9387593622; ISBN-13: 978-9387593626; Publisher: Pharmamed Press. Bridges Between Tradition and Innovation in Ethnomedicine-2011 by Maria Costanza Torri and Thora Martina Herrmann. Publisher: Springer; ASIN: B00F8KE8Q4. Medicinal Plants in India: Conservation and Sustainable Utilisation in the Emerging Global Scenario-2006 by Pradeep Kumar. ISBN-10: 8121105374; ISBN-13: 978-8121105378. Publisher: Bishen Singh and Mahendra Pal Singh. Medicinal Plants In India, 2 Vols -2002 by Pullaiah T. ISBN-10: 8187498579; ISBN-13: 978-8187498575; Publisher: Daya Publishing House.

## Conclusion

Thus, potential use of heterogeneous phyto-antibiotics may be possible in India as well as in other poor nations to curve co-morbidity of corona virus and AIDS infections. Herbal medicine is non-toxic, cheap and biocompatible. Green antibiotics is a new area where India has strong presence as our Sanskrit books like Charaka Samhita and Atharva Veda give some ideas of plant remedies. In truth, green revolution has started worldwide. *Suregada multiflora*, *Cassia fistula* and *Jatropha gossypiifolia* trees have strong presence of active phyto-chemicals and must be preserved for the treatment of MDR infections. Never-the-less our old traditional spices like derchini and labanga must be used to curve MDR infections.

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