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Review

Stress-driven discovery in the natural products: A gateway towards new drugs

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ABSTRACT

Elicitation by chemical means including heavy metals is one of a novel technique for drug discoveries. In this review, the effect of heavy metals on animal, plants and microorganisms for the production of novel compounds with the unique structures has been discussed. The number of parameters such as metal concentration, type, dose, treatment schedule, duration of metal exposure, and nutrient composition are significant factors altering the secondary metabolites production. The detailed illustrated diagram representing the mode of action of metal stress has also been discussed. This is the first article reporting all the novel compounds produced from plants and microorganisms in response to metal-stress with their pharmacological potential. This new technique opens the new way for drug discovery from natural products.

1. Introduction

The entire humankind is reliant on terrestrial and oceanic life as an origin of carbohydrates, proteins, vitamins, medicines, food, and shelter. Terrestrial and marine life has been scrutinized for their nutritional and valuable constituents for decades over decades. A distinct group of organic compounds which were produced by plants and oceanic organisms to facilitate intercommunication with the biotic habitat and establishing the defense mechanism is called “secondary metabolites” [1,2].

Terrestrial and oceanic plants and microorganisms react and adjust directly to the fluctuations in the concentrations and availability of metals within their habitat [3,4]. Thanks to the improvement of techniques of molecular biology and chemical biology that implies that abiotic factors such as heavy metals may switch the production of natural compounds by modifying the attitude of secondary metabolism [1]. The processes of induction of secondary metabolism are very diverse and complex, and the mode of action by which abiotic stress factors like heavy metals implement their effects on secondary metabolites is not yet fully explained and realized.

Natural products always performed a role of a basic frame of medicine throughout human history. In the modern era, the value of naturally derived drugs has been tremendously elevated because of their efficacy and safety as compared to synthetic drugs. Although the potential of marine organisms and terrestrial plants to produces novel secondary metabolites seems limitless, because of few major obstacles that hinders the transformation of biomolecules into medicines. One of the major obstacle during analyzing secondary metabolites of these medicinal important drugs are genes clusters or non-activated biosynthesis pathways [4,5]. Such gene clusters become entitled as sleeping gene clusters. The impact of metals in the culture medium can induce or enhance the synthesis of secondary metabolism. In modern research era of metabolomics, literature over literature is announcing the out comings of heavy metals concerning its physiology and biochemistry. Despite the physiological results of heavy metals, the results of heavy metals on bioactive components and ultra-morphological variation in distinctive parts of medicinal oceanic organisms and terrestrial plants have still been kept in dim lights. The objective of this study is to assess and address the response of heavy metal stress on the terrestrial and oceanic plants and organisms, with highlighting the

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achievements of novel medicinal compounds by an awakening of sleeping genes clusters or non-synthetic biosynthesis pathways by generating an innovative wave for drug discovery by stress strategy.

2. Heavy metals stress

The metals with a density greater than 5 g cm^{-3} are called heavy metals, but, not all of them have an importance in the field of biology. Among them, Manganese, Iron, and Molybdenum are considered as micronutrients. Nickel, Zinc, Cobalt, Copper, Vanadium, Tungsten and Chromium are toxic elements. Silver, Mercury, Cadmium, Antimony, and Lead have no role as nutrients and appear as toxic to microorganisms but some kind of tolerant by plants [6–9]. Several heavy metals are main micronutrients since they are incorporated into enzymes and cofactors (Iron Zinc, Manganese, Cobalt, Nickel, Vanadium) but in high concentrations they are still toxic because of adversary binding to enzymes and DNA, and also due to Fenton reaction by producing oxygen radicals [10]. A lot of research and literature is available describing heavy metals effects on plant morphology and physiology, but very less research has been conducted describing heavy metals effects on secondary metabolites production. Metals can enhance the production of natural compounds by regulating aspects of secondary metabolism [1,34]. Metals including Copper, Cobalt, Nickel, Zinc, and Iron have been reported to elicit secondary metabolites production in a variety of plants and microorganisms [4,5,11]. Terrestrial and oceanic plants and organisms react and adjust directly to the fluctuations in the concentrations and availability of metals within their habitat [12]. Little is known about how microorganisms interact with metals, but their interactions are generally hypothesized in three ways: the metals elicit a response in microorganisms; metals are oxidized or reduced to conserve energy in dissimilatory reactions; metals are exploited by microorganisms in assimilatory reactions [12].

3. Secondary metabolites elicited by metal stress technique

Secondary metabolites have significant practical applications in the fields of medicine, nutrition and cosmetics [13,31]. For instance, plants and microorganisms secondary metabolites have been explored for their antimicrobial, anticancer, anti-oxidant, anti-HIV and anti-parasitic activities (Table 1). Accumulations of secondary metabolites often occur in plants [14,15] and microorganisms [4,5,8,22] subjected to environmental stresses including heavy metals.

3.1. Plants and animals-derived secondary metabolites

All the novel secondary metabolites elicited from animals, plants, and microorganisms by metal stress technique have been described here. An unusual novel sesquiterpene stress metabolite anhuienol (**1**) (Fig. 1) was induced from the leaves of *Chloranthus anhuiensis* by induction of $CuCl_2$. To elicit the stress, 2% aqueous solution of $CuCl_2$ was sprayed on the leaves of *C. anhuiensis*. After applying the heavy metal $CuCl_2$ on the leaves of C. *anhuiensis*, a new spot was popped out in the metal-treated plant extract while in comparison with the control plant extract on TLC plates. As a result a new compound auhuienol was isolated in response to $CuCl_2$ stress [17]. A new phenolic diglycoside ichangoside (**2**) (Fig. 1) was produced in response to $CuCl_2$ toxicity from the leaves of *Viburnum ichangense*. The new metal elicited phenolic diglycoside was pretended to be an antioxidant activity-promoting transformation, which was hypothesized to play a key role in the antioxidative defense system of the plant [24]. The widely used heavy metal $CuCl_2$ induced two novel glucoside esters compound (**3**) and (**4**) (Fig. 1) from the fresh leaves of *Portulaca oleracea*. The two novel metals elicited secondary metabolites, compound (**3**) 9-(6-O-[(2E,6S)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-b-D-glucopyranosyloxy)-guaiacylglycerol (Fig. 2) and compound (**4**) 9-(6-O-[(2E,6S)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-b-D-glucopyranosyloxy)- syringoylglycerol (Fig. 1) with a glucose bridge, exhibited a strong antioxidant activity with IC_{50} values of 11.6 ± 0.6μM and 36.7μM ± 5.7 much stronger than those of their precursive monoterpenes [25]. A recent and modern analytical technique "Metabolomics" was used to check the all metabolites profile through UPLC-MS and NMR. For the first time the metal $ZnCl_2$ was used over marine soft corals *Sarcophyton ehrenbergi* as a chemical elicitor to evaluate its potential towards heavy metal stress and analyze the change in secondary metabolites production. The chemometric analyses of UPLC-MS results after 24 h and 48 h clearly revealed that $ZnCl_2$ with its ionic concentration of 1 mM elicited coral samples were enriched in the acetylated diterpene "sarcophytonolide I (**5**) (Fig. 1), cholesteryl acetate and sarcophine". The results showed that the quantity of sarcophytonolide I was 17-fold enhanced as compare to the soft coral samples without $ZnCl_2$ [20].

Okem et al., [15] investigated the effects of mix metals (Cd) and (Al) on *Hypoxis hemerocallidea* to examine its effect on total phenolic, flavonoids and bioactive compound hypoxoside. The phytochemical screening revealed the increase amount of phenolic and flavonoids in metal treated specimen. The bioactive compound hypoxoside skyrocketed the antioxidant (DPPH) and antibacterial (*S.aureus*) activity. The unusual piles of secondary metabolites such as shikonin [26] and also the yield of digitalin [27] were examined by treating Cu and Cd in hairy root cultures of *Brugmansia candida*, silver nitrate ($AgNO_3$) or cadmium chloride ($CdCl_2$) uplifted the yield of two tropane alkaloids, scopolamine, and hyoscyamine [18]. The most immense medicinal herb *Nigella sativa* has been tested by elicitation process to enhance its thymoquinone (TQ) and Thymohydroquinone (THQ) production along with enhanced antibacterial and antioxidant activity. The manganese chloride $MnCl_2$ has been selected as a chemical elicitor with its three different ionic concentrations (5 mg/L, 10 mg/L and 15 mg/L). The *Nigella sativa* suspension extracts with $MnCl_2$ 10 mg/L exhibited a remarkable antibacterial activity against *E.coli*, *S. aureus*, *S. Typhi* and *K. pneumoniae* with MIC value of 2.46, 2.54, 2.75 and 2.59 μg/ml as compare to other concentrations and competitive elicitors. $MnCl_2$ 10 mg/L elicitation enhanced the DPPH radical inhibition activity. The TQ and THQ were quantified by LC–MS/MS in the cultures with potent therapeutic potency revealing superlative content under $MnCl_2$ 10 mg/L elicitation. These results demonstrated the importance of $MnCl_2$ elicitation and its use for industrial scale cultivation [19].

3.2. Microorganisms-derived secondary metabolites

Two novel tyrosinase inhibitory sesquiterpenes 1β,5α,6α,14-tetraacetoxy-9α-benzoyloxy-7β *H*-eudesman-2β,11-diol (**6**) (Fig. 2) and 4α,5α-diacetoxy-9α-benzoyloxy-7β*H*-eudesman-1β,2β,11, 14-tetraol (**7**) (Fig. 2), were produced as $CuCl_2$ stress metabolites from the marine fungus *Pestalotiopsis* sp. isolated from marine algae *Sargassum horneri*, which was collected from the seashore in Wenzhou, China. The stressed culture of fungi was fermented with additionally 50 μmol/L $CuCl_2$. The compounds exhibited a tyrosinase inhibitory activities with IC_{50} value of 14.8 μM and 22.3 μM respectively [28]. A naturally novel cyclopeptide, clavatustide C (**8**) (Fig. 2) and known compound clavatustide B (**9**) (Fig. 2), was induced as a Zinc stressed metabolite from marine hydrothermal vent fungi *Aspergillus clavatus* isolated from a crab *Xenograpsus testudinatus* obtained from Taiwan. The stressed culture of fungi was fermented with additionally 50 μmol/L $ZnSO_4$. This is the first ever work reported of Zn-induced cyclic pentapeptide as a novel stress metabolite induced from hydrothermal vent. The compound suppressed cell proliferation assay against various number of cell lines in dose and time-dependent manner including gastric cancer (MGC-803), pancreatic cancer (Panc-1), colorectal cancer (SW-480), prostate cancer (PC3) and retinoblastoma (WERI-Rb-1). The cell viabilities were reduced by 65%, 74%, 63%, 67% and 78% in Panc-1, MGC-803, SW-480, WERI-Rb-1 and PC3 cells, respectively, at a concentration of 40 μg/mL after 72 h treatment [21]. Jiang et al. [5] implemented the stress-driven technique to untapped the small biomolecules from

Table 1
Secondary metabolites elicited by heavy metals.

Compound	Chemistry	Source	Part of isolation	Species	Metals	Pharmacologic actions	Effect of metals	References
Anhuienol (1)	Sesquiterpene	Plant	Leaves	*Chloranthus anhuiensis*	$CuCl_2$	–	Novel compound production	[17]
Ichangoside (2)	Glycoside	Plant	Leaves	*Viburnum ichangense*	$CuCl_2$	Anti-oxidant	Novel compound production	[24]
Compound (3, 4)	Glucoside esters	Plant	Leaves	*Portulaca oleracea*	$CuCl_2$	Anti-oxidant	Novel compound production	[25]
Hypoxoside	Glycoside	Plant	Rhizome	*Hypoxis hemerocallidea*	Mix metals ($Cd^{2+} + Al^{2+}$)	Anti-oxidant, antibacterial, Immuno-stimulant.	Enhancing the therapeutic action	[15]
Shikonin, Scopolamine and Hyoscyamine.	Alkannin, Alkaloid,	Plant	Roots	*Brugmansia candida*	Cu^{2+}, $CdCl_2$	Anti-HIV, Anti-inflammatory, Anti-spasmodic.	Enhancing the phenolic, flavonoids production along with enhancing the therapeutic actions.	[18]
Black seed	Alkaloid	Herb	Seeds	*Nigella sativa*	$MnCl_2$	Anti-biotic, anti-oxidant.	Enhancing the thymoquinone (TQ) and Thymohydroquinone (THQ) production along with elevating the antibacterial and antioxidant activity.	[19]
Sarcophytonolide I (5)	Terpene	Marine soft coral	Tissue	*Sarcophyton ehrenbergi*	$ZnCl_2$		First time enhanced production by metal stress.	[20]
Compound (6), (7)	Sesquiterpene	Marine algae	Fungus	*Pestalotiopsis* sp.	$CuCl_2$	Tyrosinase inhibitors	Novel compound production	[28]
Clavatustide C (8), B (9)	Peptide	Marine Crab	Fungus	*Aspergillus clavatus*	$ZnSO_4$	Anti-cancer	Novel compound production	[21]
Aspochracin (10)	Peptide	Marine Crab	Fungus	*Aspergillus sclerotiorum*	Cu, Cd	Insecticidal, Cytotoxic	First time compound production from marine fungus	[5]
Clavatol (11)	Terpenoid	Marine Crab	Fungus	*Aspergillus sclerotiorum*	Cu, Cd	–	First time compound production from marine fungus	[5]
Aspergstressin (12)	Polyketide-Terpenoid	Marine Crab	Fungus	*Aspergillus sp*	Co	–	Novel compound production	[4]
Cyclo-(L-tryptophyl-L-phenylalanyl) (13)	Alkaloid	Marine Crab	Fungus	*Aspergillus sp*	Co		Known compound	[4]
Cordyol C (14)	Diphenyl-ether	Marine Crab	Fungus	*Aspergillus sp*	Co	Anti-Viral	Known compound	[4]
Sydonic acid (15)		Marine Crab	Fungus	*Aspergillus sp*	Co	–	Known compound	[4]
Cyclizidine analog (16)	Indolizidine alkaloid	Marine Crab	Actinobacteria	*Streptomyces sp.*	$NiCl_2$	Anti-biotic	Novel compound production	[8]
Enterocin (17)	Peptide	Marine sediment	Actinobacteria	*Streptomyces sp.*	Co	Anti-biotic	First time and enhanced production by metal stress	[22]
Neocitreoviridin (18), 10Z-isocitreoviridinol (19), Penicillstressol (20), Isopenicillstressol (21)	Polyketide	Marine sediment	Fungus	*Penicillium sp*	Co	Anti-biotic	Novel compound production	[23]
Citreoviridin (22), Epicitreoviridinol (23), Citreoviridinol (24), Epiisocitreoviridinol (25)	Polyketide	Marine sediment	Fungus	*Penicillium sp*	Co	Anti-biotic	First time production by metal stress.	[23]
Stremycin A (26), Stremycin B (27)	Polyketide	Marine sediment	Actinobacteria	*Streptomyces pratensis*	Ni	Anti-biotic	Novel compound production	[16]

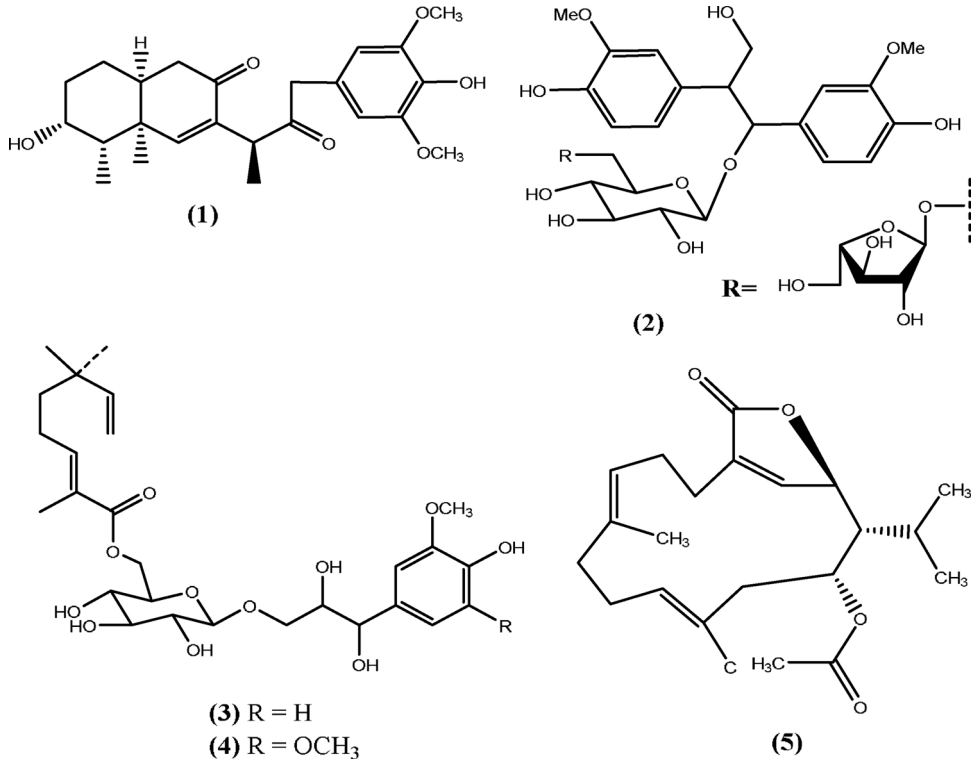

Fig. 1. Chemical structures of metal-elicited compounds derived from plants and animals.

marine hydrothermal vent fungi *Aspergillus sclerotiorum* isolated from a crab *Xenograpsus testudinatus* collected from kueishantao hydrothermal vent, Taiwan. Two known compounds aspochracin (**10**) and clavatol (**11**) (Fig. 2), were induced for the first time from marine fungi by copper and cadmium stress. The compound aspochracin exhibited insecticidal and cytotoxic activities [5]. One novel Cobalt induced hybrid polyketide-terpenoid, aspergstressin (**12**) (Fig. 2) was produced from culture broth of marine hydrothermal vent fungi *Aspergillus sp* obtained from a marine crab *Xenograpsus testudinatus* collected from Taiwan. The strain WU243 was tested against a panel of 3 heavy metals including Zinc, Cobalt and Nickel but the four new peaks only appeared at Cobalt at the concentration of 6 mM. The 4 new peaks were purified and identified as one novel compound aspergstressin and three known compounds cyclo-(L-tryptophyl-L-phenylalanyl) (**13**), cordyol C (**14**) and sydonic acid (**15**) (Fig. 2) [4].

Haferburg et al. [6] for the first time announced the heavy metals effects on secondary metabolites derived from actinobacteria. They demonstrated the awakening of sleeping genes by induction of heavy metals. 10 different actinobacteria strains were evaluated for its metal tolerance tests against Nickel and Cadmium. Some metal treated extracts displayed an enhanced antibiosis activity against *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Escherichia coli* and *Candida albicans* [6]. Shi and his team [8] discovered a novel $NiCl_2$ induced antibiotic cyclizidine analog, compound **(16)** (Fig. 2), isolated from hydrothermal vent *Streptomyces sp*. WU20 isolated from a crab *Xenograpsus testudinatus* collected from Taiwan. The new peak was elicited at all the four initial concentrations 100, 200, 400 and 800μM of $NiCl_2$ but the peak elicited at 100μM displayed a potent antibacterial activity as compare to other concentrations. Furthermore, metabolomics studies were also performed for stress-elicited antibiotic compound. This was the first example of stress derived bioactive compound originated from actinomycete. The novel compound displayed a high ratio of antibacterial activity at 100 μM nickel ions against *Bacillus subtilis* with MIC value of 32 μg/ml. A strong bactericidal compound enterocin (**17**) (Fig. 2), was produced from Co^{2+} elicited marine *Streptomyces sp*. H-1003 collected from South East China Sea. At the level of Co^{2+} 2 mM concentration ions a new peak was discovered in the culture medium which was totally absent in the other concentrations and blank culture. Furthermore, the compound enterocin's production was enhanced with using response surface methodology. The optimal condition was set containing culture of marine actinobacteria H-1003 with addition of Cobalt 2 mM ions in optimized Gause's medium having starch 20 mg for 10 days at 180 revolution/min. Under optimized condition. enterocin production was enhanced with a value of 5.33 mg/L, which was totally absent at H-1003 control and much higher than competitive conditions. This was the first ever work reported for production of enterocin compound by metal stress technique and enhanced enrichment of metal elicited compound. The enterocin exhibited a strong bactericidal activity against *S. aureus* with MIC value of 62.5 μg/ml, *E.coli* with MIC value of 31.25 μg/ml and *B. subtilis* with MIC value of 31.25 μg/ml [22].

Eight polyketide cryptic antibiotics containing four novel compounds Neocitreoviridin (**18**), 10Z-isocitreoviridinol (**19**), Penicillstressol (**20**), Isopenicillstressol (**21**) (Fig. 2) and four known compounds citreoviridin (**22**), epicitreoviridinol (**23**), citreoviridinol (**24**) and epiisocitreoviridinol (**25**) (Fig. 2) have been produced from a cobalt (6 mM)-stressed marine derived fungus *Penicillium* sp. All the compounds have been produced by HPLC-guided isolation. Initially six metals containing manganese, cobalt, nickel, chromium, zinc and cadmium with their initial concentrations 0.5, 1, 2 and 4 mM with a blank fungi *penicillium* sp. were set to check their tolerance towards metals. Due to the absence of new peaks in HPLC chromatogram of stressed-culture, the metals concentration were increased and finally on cobalt 6 mM ions 8 new peaks were observed. The structures of new compounds (20) and (21) are the first example of 2, 5-dioxabicyclo [2.2.1] heptane pyrone backbone containing a migrated polyene chain. The novel compounds (19–21) displayed a prodigious antibiotic activity against *methicillin resistant Staphylococcus aureus* (MRSA) with MIC values ranging from 0.5 to 1 μM. The known compounds (23, 25) also displayed a potent activity against MRSA with MIC values ranging from 1 to 4 μM. Additionally, the compounds (18, 20, 21) also displayed a strong antibiotic activity against *P.aeruginosa* around MIC values of 4μM following by compounds (19, 22) with the MIC values of 8μM.

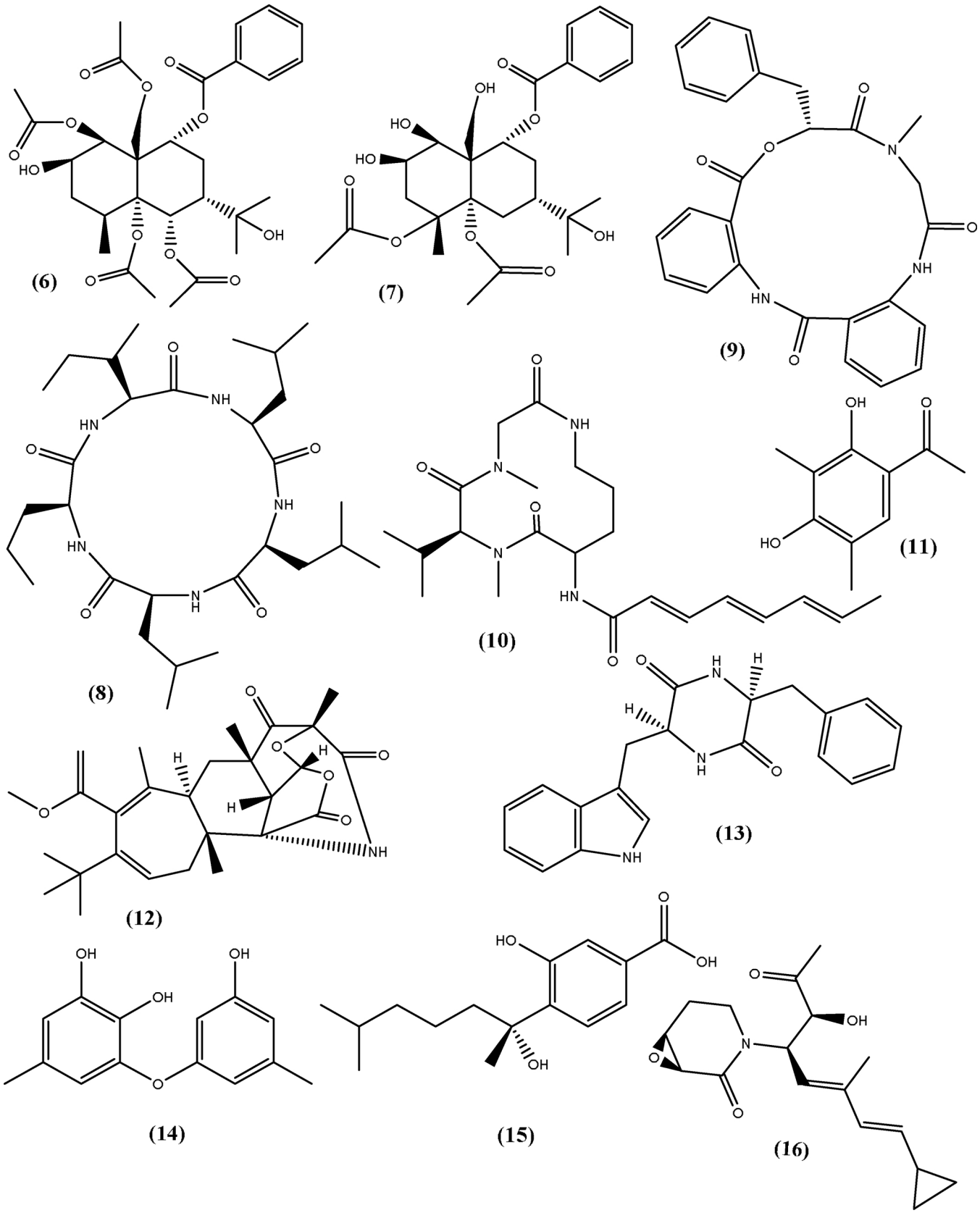

Fig. 2. Chemical structures of metal-elicited compounds derived from microorganisms.

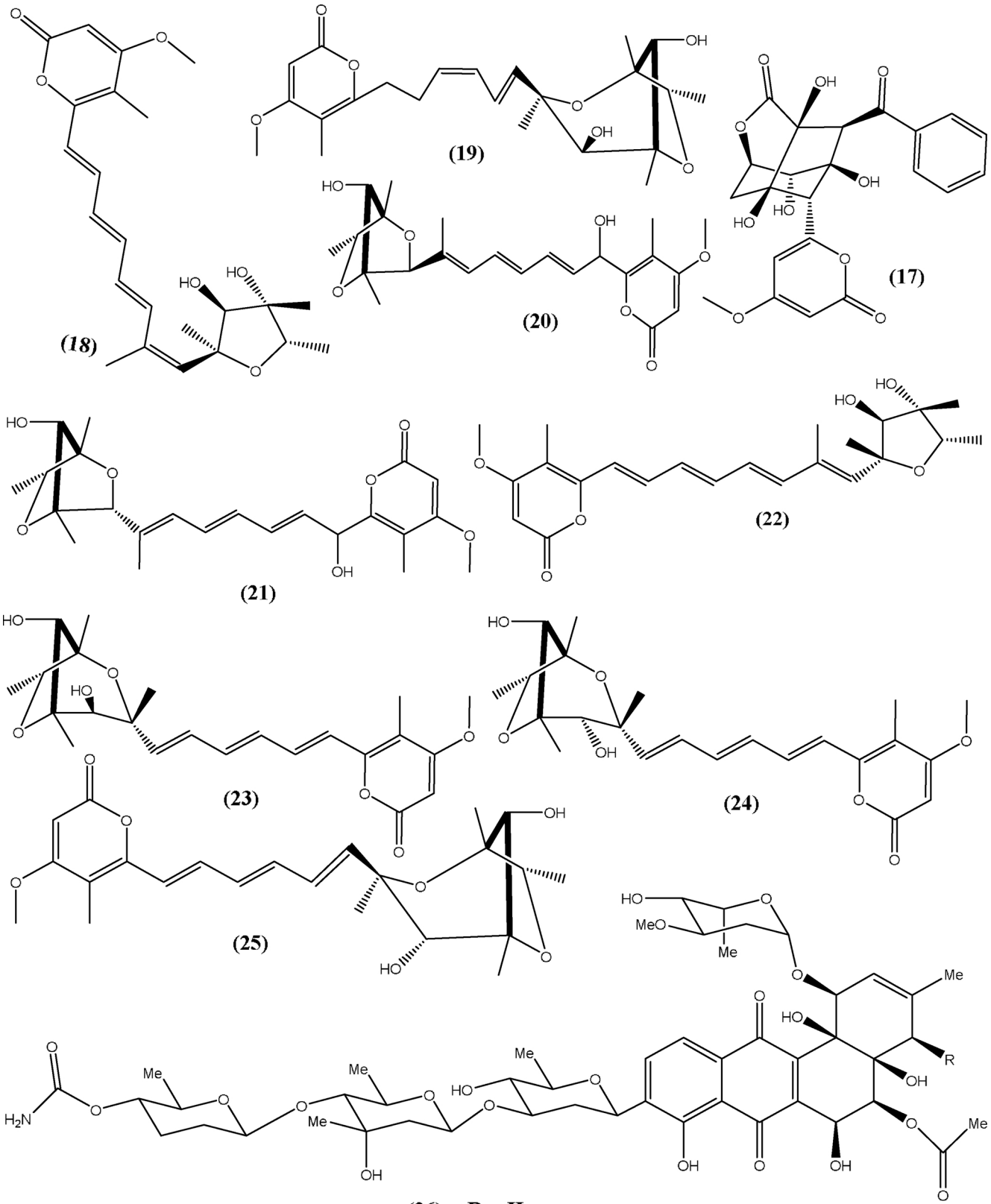

Fig. 2. (*continued*)

Furthermore, the mechanism of action of novel compounds have been hypothesized that, novel compounds exhibiting antibacterial activity might act on RNAP, which in result disturb the interaction between RNAP and DNA, resulting in failure of formation of active open promoter complex during stage of transcription [30]. Finally, with a plausible mode of action it can be hypothesized that these compounds could be perceptible transcription inhibitors [23]. Two novel polyketides, Angucycline type antibiotics Stremycin A (**26**) (Fig. 2) and Stremycin B (**27**) (Fig. 2) were isolated from marine derived actinobacteria *Streptomyces pratensis* with its induction with Nickel ions at concentration of 100μM. Both the novel compounds were deactivated in its original control culture without addition of Nickel ions. The new peak was displayed prominently in the mixed culture of *Streptomyces sp* and Nickel ions. The carbon core of both novel compounds is totally different from other compounds purified from same strain. The new compounds also exhibited its antibacterial activity against *Bacillus subtilis* with MIC value of 8–16 μg/mL [16].

4. Parameters of metal stress

Currently, chemical elicitation especially heavy metal stress has been victimized worldwide to induce secondary metabolites synthesis or to elevate the production of therapeutically active components in plants and microorganisms [1,22]. This carved up a new mine of research that could have esteemed economic advantages for the pharmaceutical enterprise. A no of specific parameters such as selectivity and concentration of heavy metals, the time span of heavy metal exposure, life span of culture, growth regulation, cell line, structure of nutrient composition, and features of cell wall materials are also fundamental factors driving the prospering production of secondary metabolites [32]. The affection of these parameters regarding metals effects on secondary metabolism is discussed here.

4.1. Heavy metal concentration

The concentration of heavy metal plays a crucial role in elicitation technique. By applying the eminent dose of heavy metals has been reported for causing hypersensitive response driving to cell death, where as an ideal level was required for induction [7,8,22]. At 2% aqueous solution of $CuCl_2$ spray on plants have emerged an additional spot in TLC plates which was totally absent in control and finally leads to a novel compound [24]. Two additional novel compounds have been produced by spraying of 2% aqueous solution of $CuCl_2$ on stems and leaves of a plant. As compare to the stressed samples (Stems and leaves) these two novel compounds were totally disappeared in the control sample (Stems and leaves) [25]. For the first time the metal stress technique was applied on soft corals to check its ability towards heavy metals. The production of acetylated diterpene, sarcophytonolide 1 was elevated about 17 fold after 48 h at 1 mM $ZnCl_2$, while at 0.1 mM concentration there was no specific enhancement in the sarcophytonolide 1 production, suggesting an activation of specific acetyl transferases [20]. On exposure of heavy metal Nickel towards marine actinobacteria have lead a novel potent antibiotic. The novel antibiotic structure was totally unique as compare to its control strain compounds. The novel compound was produced in all the four initial concentrations (0.5, 1, 2 and 4 mM) but the high level of compound productivity was observed in 1 mM Nickel. The unique carbon core of this novel antibiotic popup's the metal induction in combination with metabolic analytical technique in accelerating the mining of novel natural medicines [8]. One of the marine fungi was examined to check its ability towards heavy metal. At the initial 4 concentration of heavy metal cobalt there was no difference between stressed culture and control, exhibiting fungus power to resist metal stress. After increasing the concentration of metal up to 10 mM, the new peak was observed in 6, 7 and 8 mM of cobalt and at the level of 10 mM the secondary metabolites production was totally demolished because of toxicity level of heavy metal and the highest productivity was observed in 6 mM of cobalt. The new peak at 6 mM of metal induction with marine microorganism leads to a novel compound [4].

4.2. Duration of metal exposure

The duration of metal exposure to the plants, animals or microorganism have a very significant effect on secondary metabolites production. The marine actinobacteria *Streptomyces.sp* broth culture was treated with Cobalt 2 mM for 2, 4, 6, 8, 10, 12 and 14 days. The maximum level of enterocin, antibiotic production was recorded on the 10^{th} day with the metal concentration of 2 mM (5.33 ± 0.11 mg/L), when the growth cycle reached to 8 days the enterocin compound production was recorded (4.85 ± 0.35 mg/L) while when the growth days exceeded than 10, there was a drastic fall in the production of an enterocin (4.12 ± 0.12 mg/L) [22]. The soft coral *Sarcophyton ehrenbergi* was treated with $ZnCl_2$ for 24 h and 48 h to access its secondary metabolite accumulation. With the elicitation of 1 mM the secondary metabolites of soft corals, sarcophytonolide I was enhanced up to 17-fold, reaching up to the sky rocketed level of 11.5 μg/mg of dry coral weight and the level of cholesteryl acetate was increased up to 3 fold after 48 h post elicitation. Simultaneously, the sarcophine level was decreased up to 4.7 fold after 48 h, while at 24 h the sarcophine level was higher than the 48 h. Intrestingly the significant effects in secondary metabolites production became evident only after 48 h for the increase in sarcophytonolide I level and cholesteryl acetate level and with the decrease in sarcophine level [20].

4.3. Nutrient composition

During the elicitation process, the selection of medium also plays a pretty crucial role and impact profoundly the production of secondary metabolites. In the broth culture of marine *Streptomyces* sp. the amount of enterocin compound was strongly affected by the culture medium. The starch level was examined in a culture medium with different concentrations starting from 12.5, 15, 17.5, 20, 22.5,25 and 27.5 gm/L. The highest amount of enterocin compound was produced at 20 mg/L reveling that nutrient composition is also one of the basic need for elicitation [22].

5. Heavy metals: actions and mode of actions

There are numerous factors which symbolically influence the production of heavy metals induced secondary metabolites, encompassing growth cycle of treated cells, the concentration of heavy metals, Time duration of the whole treatment cycle, and especially ingredients of growth medium [33]. The change in secondary metabolites may be one of the tactics of the plants and microorganisms to survive from their predators or to grow in unfavorable habitat [34]. Heavy metals are a kind of abiotic elicitors, if applied in minimum concentrations it can enhance or inaugurate the biosynthesis of distinct compounds [15]. One of the hypothesis is that the genes which are associated with the synthesis of specific secondary metabolites are may be stimulated in response to signaling pathways provoked by environmental challenges [35]. Stress-induced by heavy metals inflame the activation of the defensive mechanisms of the plants and microorganisms, which in turn cause the mutation in transcription of the genes responsible for coding for the enzymes that leads the biosynthesis of secondary metabolites [36]. Heavy metals uplift the yield of secondary metabolites when it applied to undifferentiated cells. The proposed pathway that ROS, such as ˙O2–, H2O2, ˙OH, originated during heavy metal exposure which in turn formulate lipid peroxidation and sequentially produce the formation of highly active signaling compounds (Fig. 3) [37]. Peroxidation of polyunsaturated fatty acids (PUFA) in cell membrane lipids results from non-enzymatic reactions initiated by ROS or enzymatic reactions catalyzed by α-dioxygenase, peroxidases, lipoxygenases, etc. [38]. The

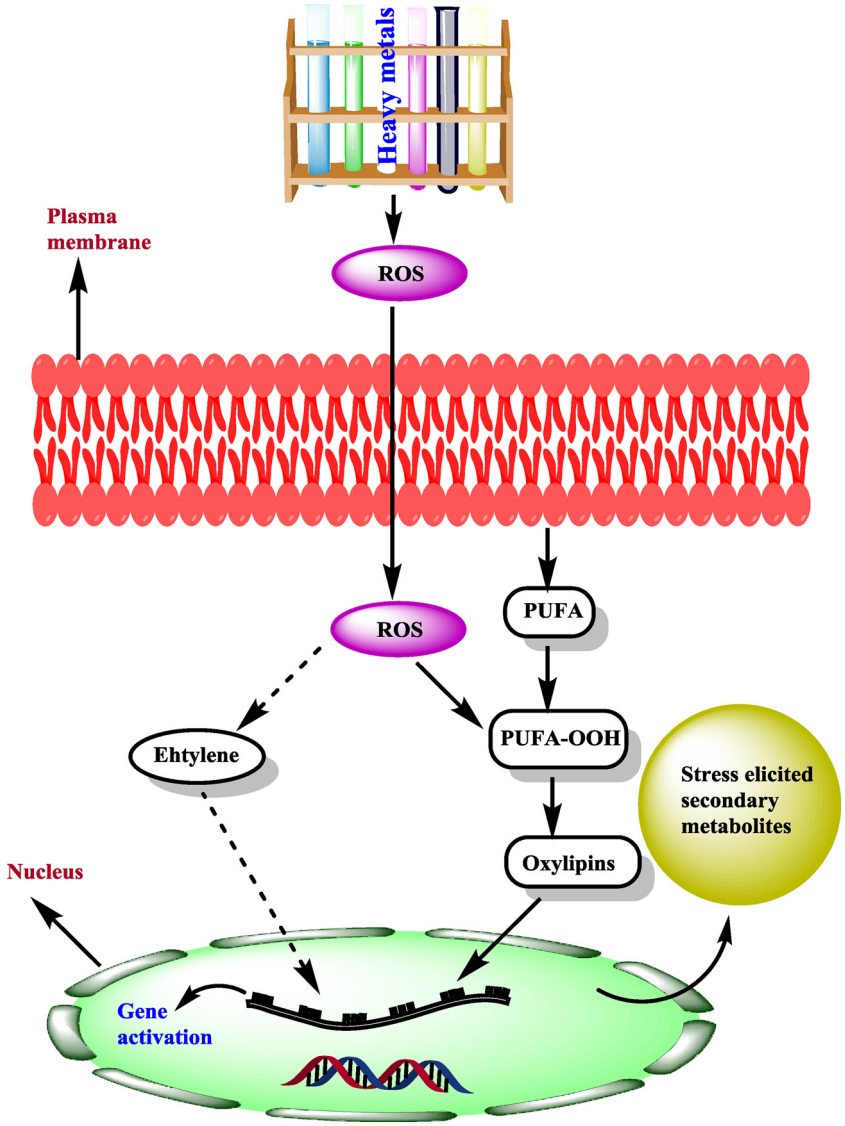

Fig. 3. A model illustrating the role of signaling pathways in heavy metal-induced enhancement of secondary metabolite production. ROS (reactive oxygen species) generated from heavy metal-induced oxidative stress leads to formation of lipid hydroperoxides, which are converted to oxylipins. Oxylipins induce the gene expression involved in the biosynthesis and accumulation of secondary metabolites. Other signaling pathways (*dashed lines*) include ethylene biosynthesis which may also play an indirect role in activation of genes involved in biosynthesis of secondary metabolites. PUFA = polyunsaturated fatty acids. −OOH = hydroperoxide.

formation of fatty hydroperoxides leads to generation of oxylipins (oxygenated fatty acids), which represent a pool of active signaling molecules that contribute to defense responses and induce expression of the genes involved in the biosynthesis and accumulation of secondary metabolites [38,39]. Non-regulated formation of oxylipins, initiated by the presence of heavy metals, may elicit secondary metabolism by generation of structurally similar or even identical compounds [34,38].

6. Conclusion

No wonder, the natural products had led a tremendous victory and success in its pharmaceuticals and nutraceuticals, but after the midth of the 20th century, the natural products are started to sink because of its lack of novel discoveries. The synthetic compounds inflamed their era because of their tremendous pharmacologic results. There were many obstacles behind the decline of natural products era, including such as a decrease in the discovery of novel natural carbon cores with its incredible new pharmacological activities, the re-isolation of already identified compounds, un-culturable environment, poor methods of structure identification and purifications e.t.c [2,31]. The new tactics and procedures are required for natural products-driven drug discovery that might reinitiate the chemical libraries with its unique carbon skeletons and its promising therapeutic activities.

The new methodologies and modern techniques and equipment should be used to improve the decline of natural products production such as culturing the un-culturable items by making its environment favorable by optimizing the laboratory conditions, genome sequencing, metabolomics, co-culture and using of different biotic and abiotic elicitors to untap the cryptic biomolecules [29]. As explained in this article, stress-driven discovery by using heavy metals as an elicitor, has established its impact on the secondary metabolism of marine organisms and terrestrial plants and leads to the production of several novel compounds. More-over in depth, searching for effective heavy metals as elicitors with their perfect concentrations have a prodigious result on oceanic organisms and terrestrial plants which in turn leading up for sparking unexpected natural products. This review built a new road for the researchers who want to isolate the new natural products from stress-driven discovery.

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