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The Chemistry Of Natural Product: Plant Secondary Metabolites

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Abstract: Natural products are those chemical compounds or substances that are isolated from living organism. It can be in form of primary or secondary metabolites. Plant secondary metabolites are organic compounds or phytochemicals that are not directly involved in the normal growth, development or reproduction of the plant. These secondary metabolites are classified into three namely; Terpenes, Phenolic compounds and Nitrogen-containing compounds. Their biosyntheses are derived from primary metabolism pathways, which include tricarboxylic acid cycle (TCA), methylerithrotol phosphate (MEP) pathway, mevalonic and shikimic acid pathway. They can be extracted from plants using organic solvents and modern separation techniques to get the analyte of interest. Their economic importance include their role in antimicrobial, pharmaceuticals, plant defence against herbivory, fragrance, stimulants, toxicity, attractant, plant breeding, physiological stress response, and allelopathtic effect.

Keywords: Metabolites, Phytochemicals, Natural products, Biosynthesis

INTRODUCTION

Natural products are chemical compounds or substances isolated from living organism. [3]. The chemistry of the natural product include their biosynthesis, extraction, identification, quantification, structural elucidation, physical and chemical properties and reactions They are produced by the pathway of primary or secondary metabolism [9]. Metabolism is defined as series of enzyme catalyzed biochemical reaction or transformation occurring within the cells of an organism which are mainly required for its growth, development and for proper response to its environment[29]. Metabolism can be in form of anabolism or catabolism. Metabolites are the intermediate or products of metabolism, the term metabolites is usually restricted to small molecules [28]. A primary metabiltes is directly involved in normal Growth, development and reproduction. Example carbohydrate, protein, fat and oil, alcohol e.t.c. Secondary metabolites are not directly involved in growth, development and reproduction of an organism, but they have an ecological function. Plant secondary metabolite can be found in the leaves, stem, root or the bark of the plant depending on the type of secondary metabolite that is been produced[14]. The most bioactive secondary metabolite are the Alkaloids, Tannins, Flavonoids and Phenolic compounds[14]. Many of these secondary metabolites are indigenous plant use as food, spices and herbs[21]. Secondary metabolites differ from primary metabolite in having a restricted distribution in the plant kingdom. That is, particular secondary metabolite are found in only one plant species or related group of species, where as primary metabolites are found throughout the plant kingdom. For many years these compounds were thought to be simply functionless end products of metabolism, or metabolic wastes. studies of these substances was pioneered by organic chemist of the nineteenth and early twentieth centuries who were interested in these substances because of their importance as medicinal drugs,poison,flavor and industrial material[28]. Only 5% to 15% of plant species have been chemically analysed so far [4]. Unlike the primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long term impairment of the organisms survivability or aesthetics, or perhaps in no significant change at all. Secondary metabolite are often restricted to a narrow set of species within a phylogenetic group[28]. Plant secondary metabolites are generic term used for more than 30,000 different substances which are exclusively produced by plants .the importance of these substances has only recently been discovered by scientists. secondary metabolite carry out a number of protective functions in the human body, it can boost the immune system, protect the body from free radicals, kill pathogenic germs and much more keep the body fit .

CLASSIFICATION OF PLANTS SECONDARY METABOLITE

Plants secondary metabolites can be divided into three chemically distinctive groups namely

- Trepenes
- Phenolic compounds
- Nitrogen containing compounds

TERPENES

This constitutes the largest class of secondary product. they are also called terpenoids.the diverse substances of this class are generally insoluble in water. all terpene are derived from the union of five carbon atom that have the branched carbon skeleton of isopentane.the basic structural element of terpene are sometimes called isoprene unit because terpene decompose at high temperature to give isoprene.terpene are toxic and feeding deterrents to many plants feeding insect and mammals. Thus they appear to have important defensive role in the plant kingdom[10]. A derivative of terpene called saponins are steroid and triterpene glycoside, so named because of their soap like properties. another derivative of terpene is caretinoids

which give the yellow, red and orange colour in some plants like carrot.examples of plant that contain terpenoids include *polypodium vulgare*,*Digitalis spp,*pine and fir, peppermint plant,lemon,basil, sage,corn,*Gossypium hispida* (cotton),wild tobacco[16].

PHENOLIC COMPOUNDS

Plants produce a large variety of secondary product that contains a phenol group- a hydroxyl functional group on an aromatic ring. These substances are classified as phenolic compounds. Plant phenolic are a chemically heterogeous compound, some soluble only in organic solvents, some are water soluble, while others are insoluble polymers. Some simple phenolic are activated by ultra violet light.phenolic are wide spread in vascular plants and appear to function in different capacities. The derivatives of phenolic compounds include simple phenyl propanoid, benzoic acid derivatives, anthocyannin, isoflavones, tannins, lignin, and flavonoid compound beginning with phenyl alanines. Lignin is generally formed from three different phenyl propanoiod alcohols namely,coniferyl,coumaryl,and sinapyl[5].The flavinoids are one of the largest classes of plant phenolics,the basic strcture contain 15 carbon arranged in two aromatic ring connected by a three carbon bridge[28]. The basic function of the flavonoids is for pigmentation and defence.the red,pink,purple and blue colours observed in plants parts are as a result of anthocyannin.the purple colour of *commelina commenis* was found to consist of a large complex of six anthocyannin molecule[17]. For example *Arabidopsis thaliana* mutant that lack flavonoids,are much more sensitive to uv-B radiation then the wild type individual and the grow very poorly under normal condition[20]. Tannins were first used to describe compound that could convert raw material hides into leather in the process of tanning.tannin are generally toxic that significantly reduce the growth and survivorship of many herbivores when added to their diets. tannins can be seen in fruits like apple, black berries,tea and red wine[28]. Tannins are mainly constituent of woody plants especially heart wood. Some derivatives of tannin include Gallic acid.

NITROGEN-CONTAINING COMPOUNDS

A large variety of secondary metabolites have nitrogen in their structure .these include the alkaloids, cyanogenic glucoside, glucosinate[28]. Alkaloids are large family of more than 15,000 nitrogen containing secondary metabolites found in approximately 20% of the species of vascular plant.the nitrogen atom in these substances is usually part of the heterocyclic ring, a ring that contain both nitrogen and carbon atom. They show striking pharmacological effect on vertebrate animal.as their name would suggest, most alkaloid are alkaline, at pH value commonly(7.2). The first medically useful example of an alkaloid was morphine, isolated in 1805 from opium poppy *papaver somniferum*[8]. The role of alkaloid in plant has been a subject of speculation for at least 100 years. Alkaloid were once thought to be nitrogenous wastes. most alkaloid are now belived to function as defence against especially mammals, because of the general toxicity and deterrence capacity[11]. One group of alkaloid, the pyroolizilne alkaloid illustrates how herbivore can become adapted to tolerate plant defensive substance and even use them in their own defence[11].

Table 1: *MAJOR TYPES OF ALKALOIDS AND THEIR EXAMPLES*

TYPE	EXAMPLE	PLANT SOURCE	USES
Pyrrolidine	Hygrine	Leaves of *peruvian coca* shrub	Stimulants, depressant.
Tropine	Atropine, cocaine	*Atropa belladonna*	Anidote of poision
Piperidine	Coniine	Bark of bomegranate,oil of hemlock. *conium maculatum*	Poison (paralyses of motor neuron)
Pyramidine-pyridine	Nicotine	Tobacco leaf *Nicotina tabacum*	Respiratory stimulation.
Quinoline	Quinine	Cinchona tree	Treatment of malaria
Isoquinoline	Codeine,morphine	*Papaver somniferum*	Analgesic, treatment of cough
Indole	Strychnine,reserpine,psilocybin	Seed of *nuxvomica strychnos,clavicept purpurea*	Rat poison, treatment of eye problem, treatment of hypertension,hal-ucinogen
Pyridine-piperidine	Anabasine	*Anabasine aphyllan*	

Source: *(Taiz and Zeiger, 2005)*

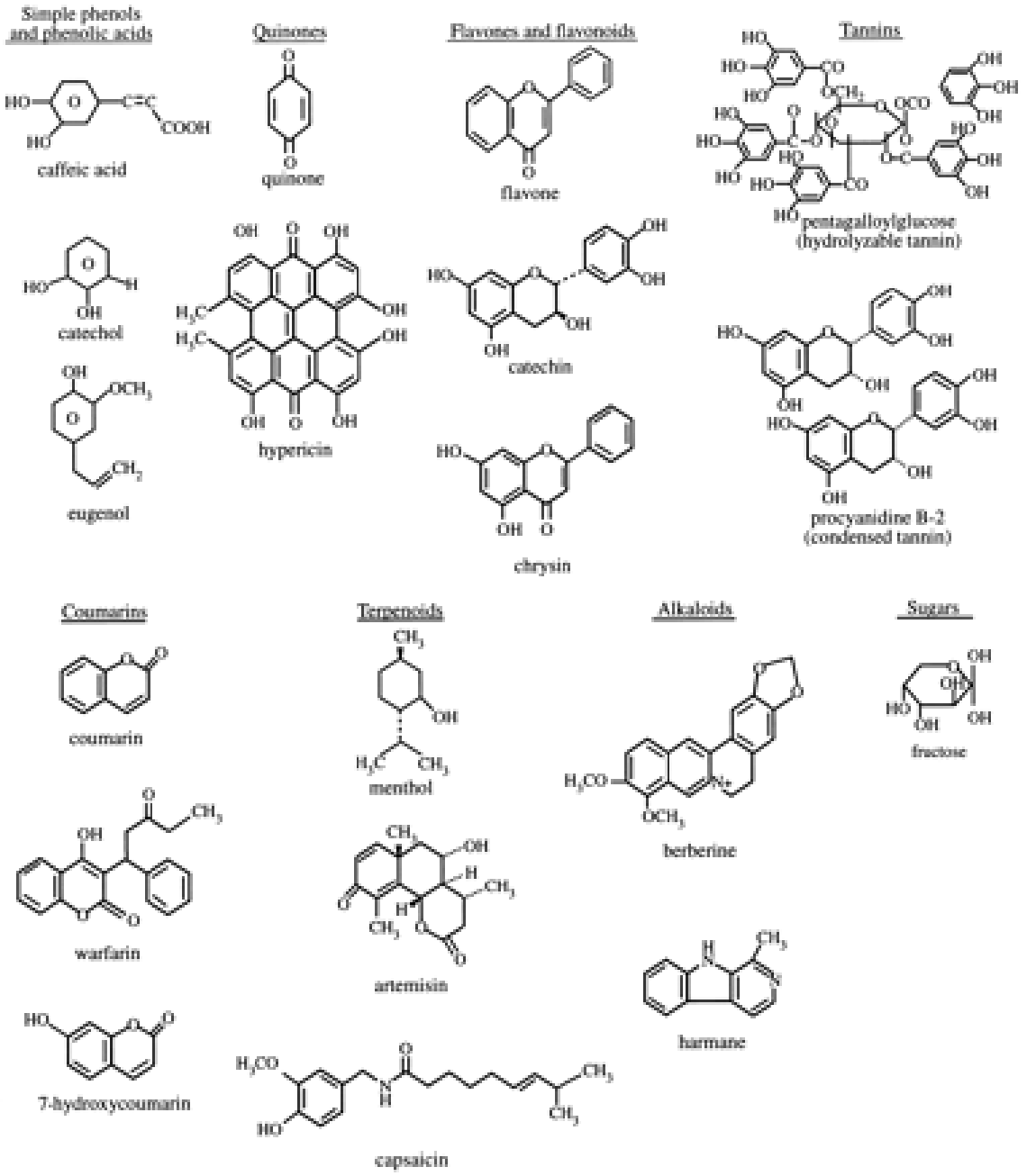

Figure 1: *Some Plants Secondary Metabolites and their structures*
Source: *(Edeoga et al., 2005)*

BIOSYNTHESIS OF SECONDARY METABOLITES

The biosynthesis of the various three classes of secondary metabolite varies depending on the class involved. The terpenes are biosynthesized from primary metabolite through the pathway of mevalonic acid and methyerithrotol phosphate[28].In mevalonic acid pathway, three molecule of acetyl-COA are joined together stepwise to form mevalonic acid. this key six-carbon intermediate is then pyrophosphorylated,decarboxylated and dehydrated to yield isopentenyl diphosphate(IPP).The IPP is the activated five carbon building block of terpenes.recently,it was discovered that IPP also can be formed from intermediate of glycolysis or the photosynthetic carbon reduction cycle via a separated set of reactions called the methylerythritol phosphate(MEP) pathway that operates in the chloroplast and other plastid[28]. Although all the details have not yet been elucidated,glyceraldehydes-3-phosphate and two carbon atoms derived from pyruvate appear to combine to generate an intermediate that is eventually converted to IPP. In biosynthesis of phenolic compound, two basic pathway are involved, the shikimic acid pathway and the malonic acid pathway[28]. The shikimic acid pathway convert simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acid[11]. One of the intermediate is shikimic acid, which has given its name to this whole sequence of reaction. The well known broad spectrum herbicide glyphosphate kill plant by blocking a step in this pathway.

the most abundant classes of secondary phenolic compound in plant are derived from phenylalanine via the elimination of an ammonia molecule to form cinnamic acid[28]. This reaction is catalyzed by phenylalanine ammonia lyase(PHL) perhaps the most studied enzyme in plant secondary metabolite. For the nitrogenous compound, the pathway is the formation of aliphatic amino acid through the TCA cycle.

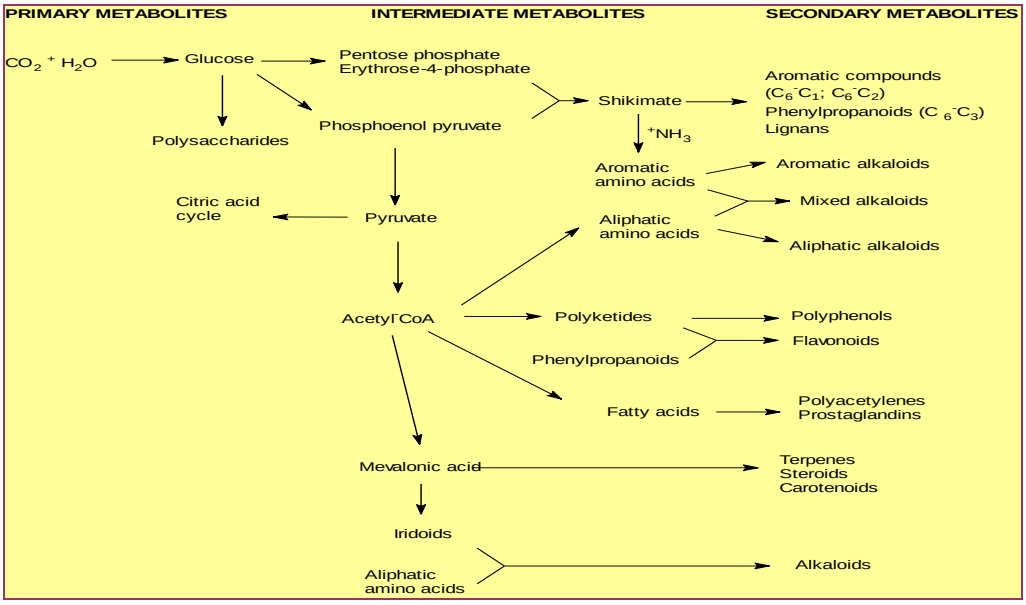

Figure 2: *Generalized plant metabolic pathway*
Source: *(Bahl et al., 2007)*

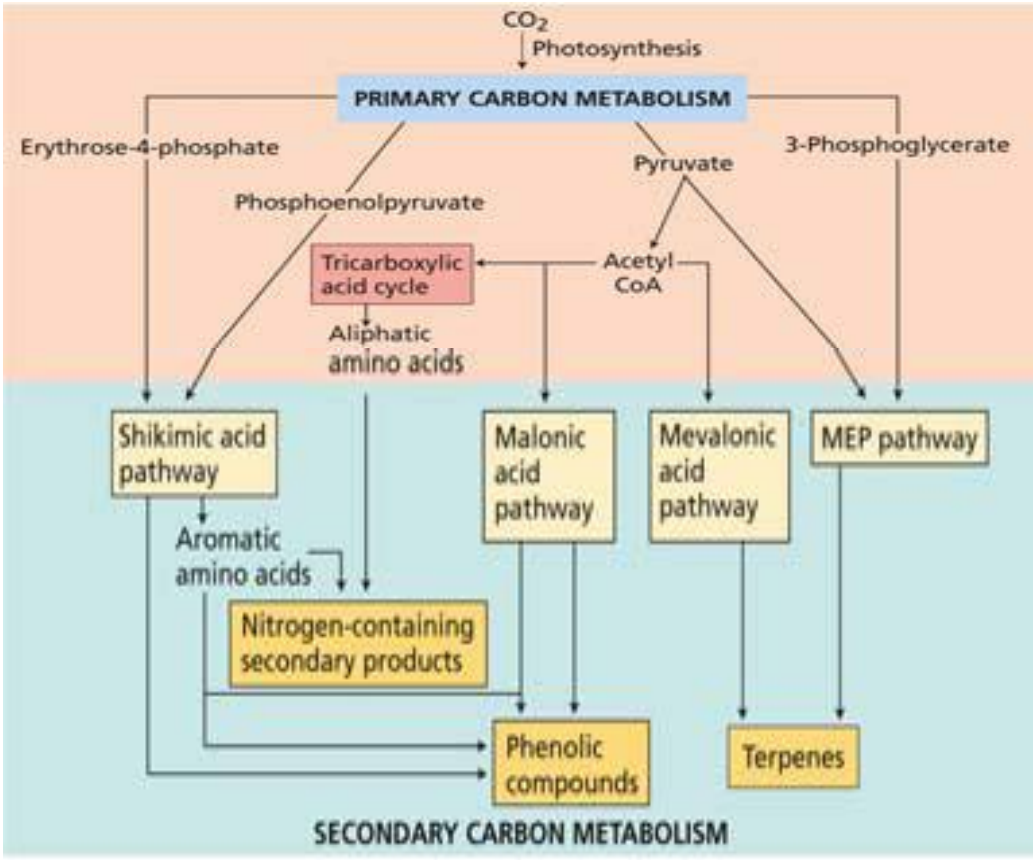

Figure 3: *A simplified view of the major pathways of secondary metabolite biosynthesis*
Source: *(Taiz and Zeiger, 2005)*

QUALITATIVE ANALYSIS OF SECONDARY METABOLITES

For the extraction alkaloid, the plant material is macerated, if the material is rich in fat, it is first extracted with ligroin or petroleum ether for their removal. The plant residue is then extracted with methanol and the cellulosic material separated by filtration. The filtrate is evaporated to give the crude plant extract. This is then dissolved in dilute acid and extracted with ether. The acid solution of the analyte salt is then basified and extracted with ether. Evaporation of ether solution gives a solid mixture of fractional crystallization for separation into individual pure pure analyte. In modern practice, isolation is effected by column chromatography. any part of the the plant may contain active component, for instance, the root of ginseng plants contain the active saponins and essential oils,while eucalypus leaves are harvested for their essential oil and tannin. Some plants, such as the balsam popular, yield useful substance in their bark, leaves and shoot[28].For alcoholic extraction, plant part are dried, ground to a fine texture, and then soaked in methanol or ethanol for extended periods. The slurry is then filtered and washed, after which it may be dried under reduced pressure and rediscover in the alcohol to a determined concentration. When water is used for extraction, plant are generally soaked in distilled water blotted dry, made into slurry through blending and then straned or filtered. The filtrate is centrifuged multiple times for clarification.

Table 2: QUALITATIVE ANALYSIS OF PLANT SECONDARY METABOLITES AND THEIR USES.

Species	Family	uses	Alkaloid	Tannin	Saponin	terpene	Flavenoid
Cieome nutidosperma	Caparaceae	Ear cure	+	+	+	_	+
Emilia Coccinea	Asteraceae	Treatment of fever	+	+	+	+	+
Euphorbia heterophylla	Euphordiaceae	Treatment cough	+	+	-	+	+
Sida acuta	Malvaceae	Stop bleeding	+	-	_	+	+
Tridax procumbens	Asteriaceae	Stop bleding	+	+	+	-	+
Richardia bransilensis	Rubiaceae	Treatment of boil	+	+	+	+	+
Citrus sinensis	Rutaceae	Anti fungi	-	-	-	+	+
Carica papaya	caricaceae	Anti-malaria	+	-	+	+	+
Cinchona sp		Anti-malaria	+	-	-	-	-

+ present, - absent ***Source:****(Edeoga et al., 2009*

Table 3: QUANTITATIVE ANALYSIS OF PLANT SECONDARY METABOLITES

PHENOL AND BENZOIC ACID DERIVATIVES (Spectrophotometry)	ALKALOIDS (Harbone method)	FLAVINOID (Bohm method)	TANNIN (Robinson method)	SAPONIN
Fat free sample +50ml ether +boil for 5mints. Take 5ml of soln+10ml dil H_2O+2ml NH_4Cl +conc. Amyl AlOH. Leave for 30mins for colour development and measure at 505nm with spectrophotometer.	5g sample + 200ml 10% acetic acid in ethanol, keep for 4hrs.filter and concentrate to one fourth of the original size + conc. NH_4Cl to form ppt, keep the soln to settle. Collect ppt and wash with dil NH_4OH then filter. the residue is dried and weighted.	10g sample extracted with 100ml 10%MeOH, filter and transfer to crucible for evaporation into dryness over water bath and weighed to a constant weight.	500mg sample + 50ml dil H_2O and shake for 1hr with mechanical shaker, filter, 5ml of filtrate + 2ml 0.1M $FeCl_3$ in 0.1M HCl and 0.008M Potassium ferrocyanide. Measure the absorbance at 120nm for 10mins.	Sample +100ml 20% EtOH + heat for 4 hrs and stir for 55^{o}C.filter and the residue re-extracted with ethanol, then mix and concentrate the soln + diethyl ether +shake. Recover aqueous layer + 60ml butanol evaporate to dryness. calculate the %

Source: *(Adopted from Edeoga, 2009**)***

TABLE 3: TEST FOR THE VARIOUS CLASSES OF SECONDARY METABOLITES

Test	Observation	Inference
Analyte +boil+ filter +0.1% ferric chloride	Browish green or a blue-black solution	Tannin present
Analyte +20ml water + boil +filter,filtrate + 5ml distll water +shake +3drops olive oil	Formation of emulsion	Saponin present
Analyte + 2ml acetic anhydride + 2ml sulphric Acid	Colour changes from violet to blue or green	Steriods present
Analyte +2ml chloroform + 3ml sulphuric acid	Formation of layers with reddish brown colouration	Terpenoids present
Analyte +10% acetic acid in aloh +stand for 4hrs +filter, filtrate B+conc ammonium hydroxide	Formation of precipitate	Alkaloid present
Analyte + 5ml ammonia +conc sulphuric acid	Yellow colouration	Flavonoids present

Source: *(Adopted from Edeoga et al., 2005)*

ECONOMIC IMPORTANCE OF PLANT SECONDARY METABOLITES

The secondary metabolites have a lot of economic importance in the plant breeding, plant defence, pollination, ecological effect and others.

PLANT BREEDING:

Plant breeders try to select varieties which provide maximal yields in combination with optimal quality and resistance against pathogen, herbivores and other environmental stress. one of the major problems of modern agriculture(both economically and ecologically) is its need for herbicides, insecticides and other pesticides. for instance potatoes (*solanum tuberosum*) contain steroid alkaloids as characteristic secondary metabolites which have been shown to be active against insect mammals and microorganism[18]. Since this steroid alkaloid are toxic. Alkaloids free tubers have been one goal of the domestication of potatoes. The alkaloid have been successfully reduced in tubers[31], But were maintained in the green parts. In this case natural resistance against enemies has been only partly eliminated during domestication which seems to be wise from the point of view of chemical ecology. a specialized herbivore of potato is the colorando potato beetle. Which is not repelled by low or medium concentration of steroid alkaloid such as solanine.when plant breeders selected for plant which were resistant to leptinotarsa,it turned out that these plant has elevated alkaloid content and were no longer useful for human consumption. It was recently found that plants of *solanum chaceese* which continued acetylated glycoalkaloids were highly resistant against the potato beetle. In the next step plant breeders intend to cross these varieties with *S. tuberosum* to confer the resistance to a crop species.

ANTI MICROBIAL:

Some herbs like(thyme).*thymus vulgaris* contain caffeic acid, which is effective against viruses, bacteria and fungi[6]. Phenolic compound containing essential oil like eugenol found *osimun gratucimum* is considered bacteriostatic against both fungi and bacteria[23]. *Aloe vera* contain combination of these metabolites together with latex is effective against *streptococcus,salmonella and staph aures.Anacardium pulsatilla*(Cashew) contains phenolic compounds that is salicylic acid which inhibit the growth of bacteria and fungi.*Solanum tuberrosum* contain alkaloids which inhibit the activity bacteria and fungi[22].

HEALTH AND PHARMACEUTICAL:

Secondary metabolite has been used in the formulation of various drugs like the alkaloids, that are used as anti malarial drug,anagestics and other antibiotics that are used to prevent bacteria infections and other health related issues.eg *solanum khasianum* mixed with other alkaloid may be useful against HIV infection as well as intestinal infection associated with AIDS[19]. Other compound used in pharmaceutical include atropine from Atropa sp, Scopolamine from Datura sp,quinine from chinchona sp, codeine from papaver.

PLANT DEFENCE AGAINST HERBIVORES AND PATHOGENS:

In conifers such as pine and fir, monoterpenes accumulate in resin duct found in the needles and twigs and trunks. These compounds are toxic to insects like bettle.marjorly, terpenes are generally toxic to plant herbivore and this is the mechanism some plant uses in defending itself from it pray. Also plant can cry for help in this case the feeding of the plant by the insect will make the plant to secreate secondary metabolite that will attract carnivores that will come and feed on the insect. this is called HERBIVORE INDUCED PLANT VOLATLE.eg can be seen for instance, the diamond back moth parasitoid *Diadegma semiclausm* is attracted to volatiles from *B.oleracea* plants infected with diamond back moth(*plutella xylostella*)larvae in a Y tube olfactometer[29].

TOXICITY:

Many of the plant secondary metabolite are toxic both to man and to animals example coniine from conium sp,strychnine from strychnos both are poisons both to man and animal[29]. Cyanogenic glycosides seen in cassava are toxic to man and animal. of special interest is the phytotoxicity of certain coumarins called furanocoumarins, which have an attached furan ring. These compound are

not toxic until they are activated by light. Phytotoxic furanocoumarin are especially abundant in members of the umbelliferae family including celery.these chemicals can cause cancers and skin related diseases.

FRAGRANCE, REPELLANCE AND ATTRACTANT:

Secondary metabolites terpenes are used as fragrance for insect pollination and perform making by extracting the volatile oil they contain eg can be seen in citronella, eugenol gotten from from *osmium gratusimum*.in general sense, secondary metabolite function as an attraction to animal for pollination (fragrance and color) or for seed dispersal. secondary metabolite are often not directed against a single organism but generally against a variety of potential enemies or they may combine the role oof both repellent and attractant eg(anthocyanin or volatile terpene can be attractant in flowers but are also insecticidal and antimicrobial. the blue colour of *commelina communis* (day flower) helps to attract animals for pollination of this plant.

ALLELOPATHIC EFFECT:

Simple phenyl propanoids and benzoic acid derivatives are frequently cited as having allelopathic activites .compound such as caffeic acid and ferulic acid occur in soil in appreciable amounts and have been shown in laboratory experiments to inhibit the germination and growth of many plant[30]. This may have a potential agricultural application, reduction in crop yields caused by weeds or residues from the previous crop may in some cases be a result of allelopathy.an exciting future engineered to be allelopathic to weed.it has been observed that any plant or plant product that can inhibit the growth of other plant contain phenolic compound as the secondary metabolites or phytochemicals[28].

CONCLUSION

Plants secondary metabolites or phytochemical have made scientists from divergent fields to have interest in plant biology and biotechnology, in investigating plant anew with the aim of isolating these phytochemicals and analyzing them both in vitro and in vivo to determine their effectiveness and their toxicity in whole-organism system, in other to find out their potential uses especially in the area of plant ecology for future prospect of developing of crop plants genetically engineered to be allelopathic to weed, for weed control. In plant breeding so as to get resistant varieties and improve crop yield so that there will be food for all and lastly in the area of antimicrobial for infection prevention, treatment and control. Attention to these issues could usher in a needed new era of chemotherapeutic treatment of infection by using plant derived principles and products. More and more information are available on the biological activities of natural products isolated from plants. However, very little is known about how their syntheses are regulated at the molecular and genetic level. The questions on gene expression of plant secondary products are still widely open and is one of the subject of research interest around the globe currently.

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