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A review on comprehensive and novel approach of phytochemicals from *Diospyros melanoxylon* as a bio-pesticide

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Abstract: *With the Green revolution commencing in the 1960s in India, the usage of chemical pesticides increased for the protection of different agricultural crops. All this resulted in an outstanding control of considerable pests in crops but also gave rise to major environmental and health related problems. The pests also started developing resistance against these chemical pesticides and responding to these concerns researchers moved towards biopesticides. Plants produce some phytochemicals that do not participate in their growth but plays a major role in their defence system against herbivores. The phytochemicals such as alkaloid, flavonoids, terpenoids and glycol cyanide eradicate pests with noxious odours, toxicity or repellent tastes. Many research trials have identified the use of phytochemicals as an effective and safe biopesticides. The main focus of this review article is to explore the Diospyros melanoxylon (commonly known as Tendu Tree) species for the novel phytochemicals and analysis methods involved for the identification of potential biopesticides. Moreover, it briefly describes about the future prospects for the further novel research proceedings.*

Keywords: *Bio- pesticides; Bioactive compounds; Phytochemicals; Alkaloids; Flavonoids*

1. Introduction

In the early 20th century, the idea of ideal pesticides evolved significantly and was used with a moderate level of control without any coordinate criteria as the potency of various compounds was prevalent. Therefore, removal of many pesticides from the existing market due to their unsuitable application such as high toxicity and persistence without proper substitute is not advisable. These applications gave rise to many cases of severe or incurable toxicity and other unfavourable effects such as a rise of resistance in the target species, the substitution of target species with more dangerous resistant species and adulteration of different environmental niche **[1]**. When these problems appeared, the United States Environmental Protection Agency (USEPA) and the European Food Safety Authority (EFSA) demanded that these pesticides be used appropriately and established agencies that were devoted for the protection of health and the environment from threats associated with pesticides. Currently, an increase in world population, combined with a decrease in cultivable land is putting tremendous pressure on the agriculture sector. Agriculture sector today, needs to achieve an increase in yield per hectare in a sustainable manner at an affordable cost. When the world faced similar challenges at the beginning of the 20th century, chemical pesticides came to the rescue and led the way to the green revolution.

Unfortunately, most of the chemical pesticides were found to cause undesirable effects on the ecosystem as well as on human and animal health **[2]**. Therefore, urgent demand for safe and efficient agriculture technologies is needed. The concept of pesticide today significantly urges to include (i) a high selectivity to target species but a minimal effect to non-target organisms, (ii) a low environmental persistence (readily degradable), and (iii) a high effectiveness at a low application rate to prevent the development of resistances and to avoid bioconcentration and biomagnification within the food chain **[3]**. Thus, there is a necessity for new bioactive stuffs that effectively fight pests & have minimal influence on humans, animals, as well as the environment. Living organisms and biotic substances plays an important role as crop protection agents. These class of biotic pesticides are usually referred to as "biopesticides" when they are produced using natural substances **[4]**. The USEPA defines biopesticides as "formulations derived from natural materials such as animals, plants, bacteria, and certain minerals." In this paper, the major focus is on plant extracted phytochemical which can be used as an effective biopesticides against pest. Plants have developed resistant mechanism to ward off different pathogenic microbes present in the environment through the production of antimicrobial agent for the regular killing of pathogens. Most of the metabolites which are produced by the plants for their defence mechanism are the secondary metabolites **[5]**. Some of the examples are saprinsphenyl propanoids where saponins are the triterpenes which mainly disrupts the sterol compounds present in the fungal system helps in binding the membrane whereas phenyl propanoids have the antipathogenic defence mechanism. It has also been observed that the other antipathogenic defence mechanisms are induced by infection **[6]**. Moreover, the plant Diospyros melanoxylon commonly called as "Tendu' also shows the presence of several bioactive compounds that can be used as an effective biopesticides **[7]**. In general plant cell walls have cutin, suberin and waxes along with some phenolic compounds and terpene which has some role in plant defence mechanism. The secondary compounds such as lignin, pigment (e.g., anthocyanin) and other metabolites may provide structural support in addition to their role in plant defence mechanism **[5]**. *Diospyros melanoxylon* is widely used commercial plant with important socioeconomic activity of tribals of India, especially of the states of Chhattisgarh, Odisha, Madhya Pradesh and Andhra Pradesh. It is also extensively available for extraction and detection of various compounds without stressing for geographical location **[8]**. Fortunately, biotechnology is expected to lead the way to achieve increase in food production in the 21st century by providing natural defence mechanism against pest using plant derived bioactive compound known as phytochemicals.

2. Phytochemicals

Phytochemical is a wide-ranging label which covers a variety of plant derived secondary metabolites or bioactive compounds with therapeutic activities such as antimutagenic, anticarcinogenic, anti-inflammatory and antioxidant properties. Guaadaoui *et. al.* (2014) explained the bioactive compounds as those which have "*the ability to interact with one or more components of a living tissue presenting a wide range of probable effects.*" Fig. 1 shows the characterization of major six categories of phytochemicals established upon their chemical structure and characteristics. These comprise carbohydrate, phenols, lipids, terpenoids, alkaloids and additional nitrogen containing compounds **[9]**. Phytochemicals are the non-nutritive component that plays a major role in plant growth and development. They provide protection from harmful agents such as insects, microbes and tough events such as ultraviolet irradiation and utmost temperature. They also fascinate advantageous birds and insects as a bait that promotes in germination, pollination, along with seed distribution. They also give colors and a range of flavors both pleasurable and unpleasurable when engrossed. They are uniquely found in particular parts of a plant with their amount increasing during stressful the conditions. Phytochemicals when consumed also shows beneficial effects to health **[10]**.

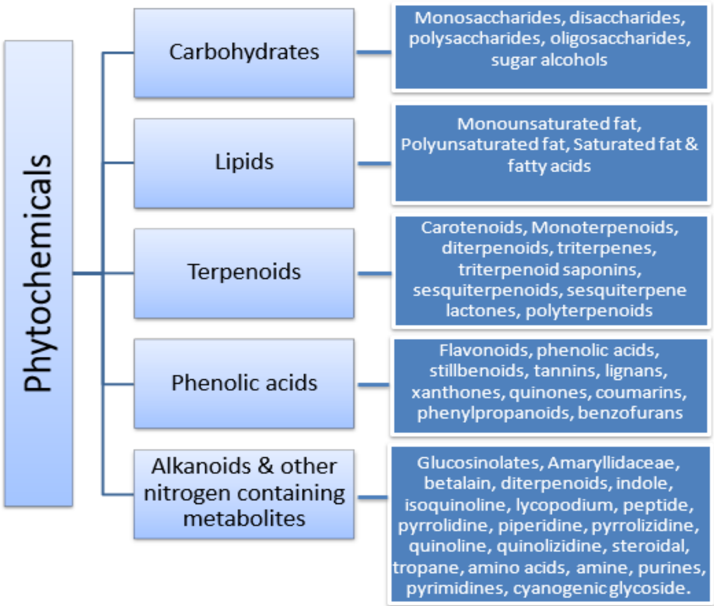

Figure 1: Characterization of major six categories of Phytochemicals

2.1 Carbohydrates:

They are defined as polyhydroxy chains of ketones and aldehydes. These are the class of natural occurring compounds and their derivatives formed from them. It is formed in plants during photosynthesis. Generally, there are two main functions of carbohydrates in plants. Firstly, they provide building blocks for plant structural components such as cellulose. Secondly, they help in plant growth by providing them energy molecules **[11]**. The cardiac glycosides derived from plants such as foxglove and lily of the valley are powerful drugs which includes digoxin and digitoxin which supports the beating rate of the heart and act as diuretics **[12]**.

2.2 Lipids:

The word lipid covers both fats and oils. Plant requires lipid as a form of storable food and for various other functions. The solid form of lipid is known as fats and its liquid form is known as oils. Lipids are used to form the structural membranes of cells that all life forms are made up of. The major part of the fats is found in the embryo and endosperm because endosperm is the source of food of the plant embryo as it grows **[13]**.

2.3 Terpenoids:

Many types of terpenoids and terpenes are majorly found in resin plants such as balsam fir and conifers. They possess a strong aroma which helps the plant to repel the herbivores. Their scent is also used for the production

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of essential oils, whether for perfumes such as rose and lavender or for aromatherapy. Terpenoids are derived from acetyl-CoA and plays an important role in the plant defence mechanism **[14]**. It effectively work as an active compound in resin or as volatiles, repellents, toxins. Many terpenoids have synergistic effects upon release **[15]**.

2.4 Phenolic acids:

Phenolic acids or phenols are found in variety of classes widespread in plants. These classes include the colourful anthocyanin, the antioxidant flavonoids, the supportive lignin, tannins, etc. Phenols are produced to act as a repellent against herbivores, inhibit enzymes, attract pollinators and fruit dispersers, absorb UV radiations and reduce the competition among plant neighbours **[16]**. Phenolic compounds upon ingestion can covalently bind to the enzymes of the digestive system of the herbivores and inactivate them. They can also stop the growth and development of the larvae **[17]**.

2.5 Alkaloids:

Alkaloids are often the toxic chemicals found widespread in nature and have a bitter taste. These are often regarded as efficient feeding deterrents against herbivore. These molecules are alkaline in nature and contain nitrogen in a heterocyclic ring. Alkaloids are majorly involved in defence of plants against insect herbivore **[18]**.

3. Description of Plant

3.1 Taxonomy: -

i. Scientific name: Diospyros melanoxylon

ii. Family: Ebenaceae Genus: Diospyros Species: melanoxylon

iii. Common name: Coromandel Ebony, Black Ebony, Ebony Persimmon, Kendu leaves, Tendu leaves

iv. Vernacular names: The different names in different languages includes kend, kendu (Bengali), Abnus, Kendu, Tendu, Timburni (Hindi), Abnush, Tendu (Nepali), Dirghapatraka (Sanskrit), Karai, Karundumbi, Tumb (Tamil) and lastly its trade name is Ebony.

Coromandel Ebony is a medium heighted tree or shrub up to the range of 25 m in height and 1.9 m circumference. The bark is grey brown in colour which resembles to the pelican bird peeling in rectangular scale which is an identifying feature. The name of the species melanoxylon comes from the Greek word melas (black) and xylon which means wood. The lower part of primary root is long, thick and fleshy and the upper part near ground level is woody, greyish, often swollen. The leaves are elliptical-oblong in shape and up to 35cm long in length. Leaves are commercially exploited in making beedis. The fruits are olive green in colour and are 1-, 2-, 3-, 4-, 5-, 6- or 8- seeded berries. The pulp of the fruit is yellowish, soft and sweet in nature **[19]**.

4. Extraction, Purification and Detection of Phytochemicals

The Phytochemicals are the secondary metabolites of the plant which cannot be used directly for human welfare. In order to use them they must be extracted and purified from the source plant.

4.1 Extraction of the phytochemicals

Selection of the extraction method depends on the nature of plant material and nature of desired compound. Extraction is experimental exercise because various solvents are employed at differing state such as the temperature and time of extraction. The majorly used methods for the extraction of phytochemicals have been discussed below:

4.1.1 Extraction of phenolic compounds by using various solvents

The impact of different types of solvents, such as methanol, ethyl alcohol and hexane were studied and analysed by scientists for the motive of extraction of antioxidant, from various parts of the plant, such as seeds, leaves & fruits. For isolating various phenolic compounds with a greater degree of accuracy from plants, different solvents of differing contrariness must be employed **[20]**. Moreover, solvents with high polarity such as methanol show greater efficacy as antioxidants. The ethanol extracts from the Ivorian plants show a greater concentration of phenol when compared to acetone, methanol, as well as water **[21]** (Koffi et al, 2010). There are multiple solvents which are frequently used to extract phytochemicals; scientists for the extraction of bioactive compounds usually make a dried powder of plants and remove the intervention of water at the same time. On the basis of polarity of the solute of interest, they are selected for extracting different biotic compounds from a plant. Here are some few common solvents placed on the basis of their polarity i.e., from least polar to most polar: Hexane < Chloroform < Ethyl acetate < Acetone < Methanol < Water **[22]**.

4.1.2 Microwave-Assisted Extraction (MAE)

MAE is a method to isolate phytochemicals from a broad variety of plants and natural resources **[23]**. It combines the traditional solvent extraction & microwave & therefore it is termed simply as microwave extraction. The kinetic of extraction increases when the solvent and plant tissue is heated using microwave and hence termed as microwave- assisted extraction **[24]**. The main purpose of heating the dried powdered plant material is due to the fact that there are very small microscopic traces of moisture present inside of plant cells. Due to the microwave effect, there is a rise in the temperature of the moisture inside the plant cell which leads to the evaporation & produces high pressure on the cell wall. The cell wall experiences a push from inside due to this very pressure which eventually causes the rupture of the cell. Thus, the percolation of bioactive molecules from the lysed cells occurs, which ultimately increases the yield of phytoconstituents **[25]**. The initial use of microwaves was to heat up objects by absorbing a part of the electromagnetic energy & converting that energy to heat. Generally, commercial microwave instruments have an energy output of 600-700 Watts & they operate at a frequency 2450 MHz **[26]**. This method has minimal losses of the biochemical compounds which are extracted **[27]**. Microwave-assisted extraction (MAE) is an important substitute to conventional techniques for extracting the antioxidants due to fact that it consumes less time & also reduces the extraction solvent volume **[28]**. The major goal of using this method is to use less amount of solvents that can be heated for extraction of the antioxidants from plants **[26]**.

4.1.3 Ultrasonic-Assisted Extraction

Ultrasound-assisted extraction (UAE) is widely used in diverse applications of food-processing technology to extract phytochemicals from plant materials **[29]**. Ultrasound, with frequency greater than 20 kHz, is used to disrupt plant cell walls, which helps to improve the solvent's ability to penetrate the cells and obtain a higher extraction yield. It is known to be the easiest extraction techniques as it uses common laboratory equipment such as an ultrasonic bath. Moreover, this technique is carried out by mixing a smashed sample with suitable solvent by placing it on the ultrasonic bath, while controlling the temperature and extraction time **[30]**.

4.2 Purification of phytochemicals

Plant extracts contains many different types of bioactive compounds which have polarities. For the process of identification and characterization of the phytochemicals it is very important to separate them from each other. The principle behind purification depends of separation on the basis of mass and on the basis of polarities of the phytochemical. The common techniques used in isolation of these bioactive compounds includes TLC, HPTLC, paper chromatography, column chromatography, Gas chromatography, OPLC and HPLC. These techniques help us to obtain pure compounds. The pure compounds are examined for determination of their structure and biological activities **[31]**.

4.3 Identification of phytochemicals

The extracts of the plants contain different kinds of bioactive compounds which have different polarities. Separation of these bioactive compounds for their identification & characterization is a challenging work. Various techniques are employed to get pure form of compounds. These pure compounds are then utilized for determining of structure & biological activity.

4.3.1 Fourier-transform infrared spectroscopy (FTIR)

For the identification of the functional groups present in the extracts of different plants, Fourier- transform infrared spectroscopy is an important tool. It significantly helps in determination & identification of structure of the molecule **[32]**.

4.3.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy can be used to determine the molecular confrontation in solution as well as help in understanding the physical properties such as conformational exchange, phase changes, solubility and diffusion at molecular level. It is also used in quality control and detection of purity of a sample. The conventional one-dimensional technique is generally used but sometimes the complicated structure of the molecules could be only achieved through two- dimensional NMR techniques **[33]**.

4.3.3 Mass spectrometry (MS)

Mass spectrometry is another powerful investigative tool widely used for the identification of unrevealed compounds, to interpret the structural and chemical properties of molecules and for quantifying the known compounds. It is also used to determine the molecular weight of the sample through MS spectrum. This method majorly demonstrates the structural confrontation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity; by determining both the molecular weight and a diagnostic fragment of the molecule **[25]**.

V. Evidence of Phytochemicals in the Plant

Muthiah Maridass (2008) worked on various species of *Diospyros* which was isolated and characterized experimentally. The chemical constituents isolated specifically from *Diospyros melanoxylon* species have been given below:

Table 1: Chemical Constituent of *Diospyros melanoxylon* species **[7]**

S.No	Plant species/ Plant part	Compound
1	D. melanoxylon Roxb. (Heartwood)	b-sitosterolterpenoid, Lupeol, Betulin, Betulinicacid, 2-methyl-methoxy-14-naphthaquinone, 3-methyl-8-methoxy-1, 9,naphthaquinone,2-methl-3-hydroxy-5 methoxy,and2-methyl5,6Dimethoxy-1,4 napthaquinone.
2	Leaves	b-sitosterol, Monohydroxymonocarboxylic acid,MonohydroxytriterpeneBauererys acetate, Ursolic,Betulinic acid, Baurenol,ursolicDiospyricacid,Isobanerenol, Methylbetulinate

VI. Phytochemicals as Biopesticides

Phytochemicals have been identified to work successful as biopesticides against more than 100 insects of 10 different orders (Orthoptera, Dictyoptera, Lepidoptera, Homoptera, Heteroptera, Diptera, Coleoptera, Hymenoptera, Isoptera, Thysanoptera) and other 100 non-insect pests. Although the phytochemicals are considered as stomach poison against pest, they can also act as pest repellent, disruptors of mating and sexual communication, sterilant, growth retardant, oviposition deterrents, antifeedants and lethal toxins (for pests). They can inhibit the spore germination and act as growth retardants of fungi and bacteria. They can also help in preventing entrance of root nematodes. The main advantage of using plant products (phytochemicals) as biopesticide is its biodegradable nature which doesn't let residues to stay on plants. Other features are that they are effective against insects, plant pathogen, nematodes and non-insect pest; non-polluting and compatible with other biopesticides **[34]**.

VII. Conclusion

Considering the current challenges in ever increasing cost of farm inputs, reduction in production costs, increase in productivity and hazardous environmental impact. The utilization of bioactive compounds or phytochemicals for the production of biopesticides could tremendously help in pest control management. The main interest of this over conventional chemical pesticide is that they do not have any adverse effects on non-target organism, ecosystem and human health. Moreover, phytochemicals expected to derive especially from region like bark other than heart wood and leaves of *Diospyros melanoxylon* (Tendu) could be cost-effective because of its availability and suitability at commercial level. It could also show high market demand than commodities produced with chemical means. As plant derived biopesticides includes target specificity, doesn't leave traces of residue on food, appear ecofriendly and are hoped to provide a safe eco-system. Also, it could be mixed in proper ratio with chemical pesticide to minimize the inappropriate effects. Therefore, leading its way ultimately to better food production with effective and proper nature.

VIII. Future Scope

There is wide scope and requirement in research and analysis of the phytochemicals extracted from *Diospyros melanoxylon* species with its commercial value to produce effective and environmental friendly biopesticides. The researchers are yet to explore the bark of the Tendu plant which is easily available in the region of central and eastern region of India. Moreover, the availability will not cause major hindrance in production of biopesticide once the analysis of the activities of phytochemicals extracted from bark of the plant is carried out.

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