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Isolation And Characterization Of Phytoconstituents From Fruits Of *Aphanamixis Polystachya*

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Abstract:s

The meliaceaeous plants are rich source of limuloids and used as pesticide in agriculture. *Aphanamixis polystachya* R.N. Parker (Wall.) belongs to the family Meliaceae and it is a traditional plantnative to Asia, especially China and India. It is extensively used in folklore medicine of Bangladesh, for the treatment of various ailments like in liver and spleen disorders, tumors, ulcer, dyspepsia, intestinal worms, skin diseases, leprosy, diabetes, eye diseases, jaundice, hemorrhoids, burning sensation, arthritis and leucorrhoea. According to previous studies, *A.polystachya* has been extensively investigated since the 1960s because of the anticancer, antimicrobial and antifungal, anti-inflammatory, anti-oxidant, anti-diabetic, insecticidal and hepato protective properties of the plant extracts. *A. polystachya* well known source of limonoids and terpenoids with the wide range of biological activity. *A.polystachya* have led to the isolation of many structurally active constituents like terpenoids and limuloids with apharmacological properties such as anti-feed ant, insecticidal and antioxidant activities. In our present study Photochemical investigation of fruits of hexane extract of *Aphanamixispolystachya* led to isolation of active constituents. The resulted active constituents were determined on the basis of HRMS, IR, 1D and 2D NMR data.

Key words: Antioxidant, ant diabetic and hepatoprotective.

1. INTRODUCTION:

Natural products can be defined as products of natural backgrounds which include organisms, plants animals. However, in maximum cases the term natural products denotes to secondary metabolites, minor molecules (mol.wt ,<200 amu) made by organisms that are not firmly needed for the existence of an organism. Many of the secondary metabolites from the plant origin have tremendous potential for use in treatment of various diseases.

Themetabolitesusedtodayaresteroid,diosgenin,codeine,atropine,reserpine,hyoscyamine,digoxin,scopolamine,digitoxin,pilocarpine,quinidine,quinine,artemesinin,senna glycosides, taxol, and aloe etc. The use of natural drugs is of great interest in human path physiology. From time to time, preparations from plants have achieved reputations as curatives for various diseases (chopra*et al.*,1956).

Natural products can be from any terrestrial or marine source plants(eg.,pacliataxel(taxol) from *taxusbrevifolia*),animal(eg.,Vitamins A and D from codliver oil or microorganisms(eg.,doxorubicin from *streptomycespeucethus*).

Natural products have been playing a vital role in health care for decades. Since ancient times, natural products represent the main source of compounds employed in drug discovery and development. From thousands of years nature has been a rich resource of beneficial biological agents and remarkable number of recent drugs has been resulting from natural sources grounded for traditional medicine.

1.1.TRADITIONAL MEDICINES FROM HIGHER PLANTS:

It is important resource for treatment of diseases from the minor ailments to more serious or acute diseases like cancer, malaria, tuberculosis etc[1]. Different societies from different regions of the world have systematically identified medicinal and poisonous plant forming part of their unique traditional medical systems over time[2].Such indigenous knowledge is deeply rooted in the people's culture and forms part of their history being passed on orally from generation to generation[1].

As a medicine natural product offers four vital and appreciable roles:

Serves as extremely useful natural drug.

Provide basic compounds affording less toxic and more effective drug molecule.

Modification of inactive natural products by suitable biological or chemical means into potent drugs and presently about 25% of pharmaceutical prescriptions in the united states contain at least

one plant derived ingredient. In the last century,roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.Exploration of biological active phenotype to newer and better synthetic drugs. Traditional medicine is the therapeutic experience of generation of practicing physicians of indigenous system of medicine. Traditional preparation comprises medicinal plants, minerals and organic matters etc. The ancient record is evidencing their use by Indian, Chinese ,Egyptian, Greek ,Roman and Syrian dates back to about 5000 years. About 500 plants with medicinal use are mentioned in ancient texts around 800 plants have been used in indigenous system of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments, which also forms a rich source of knowledge[3]. The various indigenous system such as Siddha, Ayurveda, and Homeopathy use several plant species to treat different ailments [4] .In India around 20,000 medicinal plant species have been recorded, but more than 500 traditional communities use 800 plant species for curing different diseases[5]currently 80% of the world population depends on plant –derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines.

1.2.NATURAL PRODUCTS FROM PLANT ORIGIN:

Medicinal plants play an important role in the development of potent therapeutic agents. plant derived drugs came into use in the modern medicine through uses of plant material is an indigenous cure in traditional system of medicine. Drug discovery begins by selecting material to be screened for medicinal properties. Natural products remain important sources of test material. Although many substances have been tested for medicinal use, more remain. If one particular plant is found to possess compounds of interest related plants found in the same geographic region become for screening .promising compounds identified in initial screenings can be chemically modified in myriad ways to check for improved activity or reduced side effects[6].

Metabolism is the complex of physical and chemical processes occurring within a living cell or organism that are necessary for maintenance of life .In metabolism some substances are broken down to yield energy for vital processes while other substances, necessary for life ,are synthesized.

Secondary metabolites are known to be synthesized in specialized cell types and at a distinct developmental stages, which makes their extraction and purification more difficult. These chemical constituents are extremely diverse. Each plant family genus and species produces a characteristic chemical category or a mixture of them and they can sometimes be used as taxonomic characters in the classification of the plants.

Primary metabolites show all pathways necessary to keep cell alive. secondary metabolites are chemicals produced by plants for which no role has yet been found in growth ,photosynthesis, reproduction, or other primary'' functions. These chemicals are extremely diverse. Many thousands have been identified in several major classes. It is assumed that plants evolved secondary metabolites due to their protective value. Many secondary metabolites are toxic or repellent to herbivores and microbes and help defend plants producing them .Their production increases when a plant is attacked by herbivores or pathogens.

2. REVIEW OF PHYTOCHEMICALS:

Plants produce a vast and diverse assortment of organic compounds, the majority of which do not appear to participate directly in growth and development .These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom .Their functions ,many of which remain unknown ,are being elucidated with increasing frequency. The primary metabolites, in contrast, such as phytosterols, aryl lipids ,nucleotides, amino acids and organic acids are found in all plants and perform metabolic roles that are essentials and usually evident. Although noted for the complexity of their chemical structures and biosynthetic pathways ,natural products have been widely perceived as biologically insignificant and have historically received little attention from most plant biologists .Organic chemists ,however ,have long been interested in this novel photochemical and have investigated their chemical properties extensively since the 1850.

2.1. Secondary metabolites- plant natural products:

Interest in natural products was not purely academic but rather was prompted by their great utility as dyes ,polymers ,fibres, glues, oils, waxes, flavouring agents, perfumes, and drugs. Recognition of

the biological properties of myriad natural products has fuelled the current focus of the field, namely, the search for new drugs, antibiotics, insecticides and herbicides. Importantly ,this growing appreciation of the highly diverse biological effects produced by natural products has prompted a revaluation of the possible roles these compounds play in plants ,especially in the context of ecological interactions .Many of these compounds now have been shown to have important adaptive significance in protection against herb ivory and microbial infection ,as attractants for pollinators and seed-dispersing animals and as allopathic agents (all chemicals that influence competition among plant species).These ecological functions affect plant survival profoundly ,and we think it reasonable to adopt the less pejorative term plant natural products to describe secondary plant metabolites that act primarily on their species.

LITERATURE VIEW OF *APHANAMIXIS POLYSTACHYA*

Meliaceae, the mahogany family, is a flowering plant family of mostly trees and shrubs (and a few herbaceous plants, mangroves) in the order Sapindales[43]. It includes 50 genera and 1400 species according to Willis. In India it is represented by 20 genera and 70 species. It is widely distributed in tropics of both the hemisphere.

Plants woody trees, leaves pinnately compound exstipulate; leaflets asymmetrical, margin serrate, inflorescence cymose panicles, flowers actinomorphic, hermaphrodite, calyx and corolla sometimes united, stamens 8 to 10, monadelphous, obdiplostemonous: annular necticiferous disc between petals and stamens, gynoecium, pentacarpellary, syncarpous, superior, fewer or multilocular with 1-2 rarely more ovules in each locule;singlestyle;fruits various – capsular or drupaceous.

Almost every part of Melia possesses some medicinal properties. The bark is a bitter tonic, astringent and antiperiodic. The bark, root bark and young fruits are used as a tonic antiperiodic and alterative. Leaves are used as poultice and applied to boils, the twigs as tooth brushes.Decoction of leaves is antiseptic and used to wash ulcers and eczema. The oil is used in rheumatism and skin diseases. Dry flowers are used as a tonic and stomachic. It is blood purifier.

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Leaves of *Aphanamixis polystachya*

Bark of *Aphanamixis polystachya*

Fruits of *Aphanamixis polystachya*

Seeds of *Aphanamixis polystachya*

Figure 2.9: Various parts of *Aphanamixis polystachya* plant

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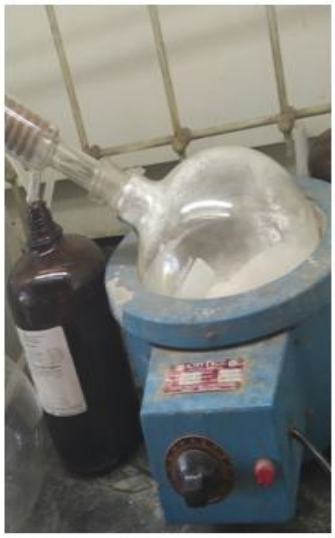
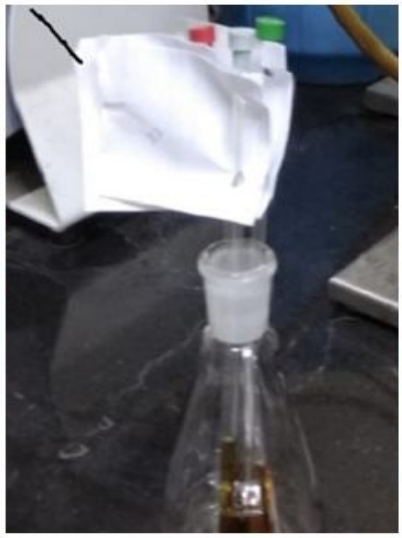
Main fraction column

Sub fraction column



Distillation Unit



NMR tubes

Rota evaporator

DISCUSSION:

- The isolated compounds in our study were screened for Antioxidant activity using $ABTS^{+\cdot}$ and DPPH scavenging assay and also for Carbohydrate/Glucose metabolism modulating activity.
- % $ABTS^{+}$ Free Radical Scavenging activity and IC_{50} Values from TABLE 5.14

Scavenging of $ABTS^{+}$ Free Radical was performed as described by Walker and Everette with suitable modifications. Ascorbic acid was used as Standard .The compound 2(Aphanamixin-B) showed moderate activity and the other compounds shows very less activity and compared with standard drug.

- % $DPPH^{+}$ Free Radical Scavenging activity and IC_{50} Values from TABLE5.15
- $DPPH^{+}$ Free radical Scavenging assay was performed as described by Yamaguchi *et al.* Ascorbic acid was used as Standard. The compounds didn't show the activity.
- % α-Glycosidase Inhibition assay and IC_{50} Values from table 5.16.The α-Glucosidase Inhibition assay was performed as described by Babu*et al.* The compound 3 (Aphananin)showed good activity, compound 1(Stigmasterol) moderate activity compared with standard drug. Acarbose is used as standard. Compound 2,4 and 5 didn't show the activity.

6.SUMMARY AND CONCLUSION

The present study describes "Isolation and Characterization of phytoconstituents from fruits of *Aphanamixispolystachya*".

The fruits were collected, authenticated, shade dried and powdered using mechanical grinder. The powdered material was extracted by soxhlet method by using polar solvent like acetone. The percentage yield of hexane extract was found to be 18% w/w in respect to the dried plant material.

The extract was subjected to preliminary phytochemical screening using standard preliminary phytochemical methods and TLC methods in which the extract was found to be rich in phytoconstituents like alkaloids, glycosides, saponins, flavonoids, anthroquinones and major part of terpenoids. Based on the column chromatography, many fractions were collected and further purified by repeated column chromatography. This was done by simultaneous monitoring of the

fractions and mixture of compounds that were eluted using various mobile phase systems.

1-6 fractions were collected and subjected to column chromatography by using solvents in increasing order of polarity i.e., hexane, acetone. Fraction -2 yielded compound-1(Stigmasterol)and fraction-3 yielded compound-2(Aphanamixin-B).

Sub fractionation of fraction-4 and fraction-5 were subjected to column chromatography by using gradient technique and a pure compound was isolated and identified as compound -3(Aphananin) and compound -4(polystanin).

Fraction-6 by washing solid a pure compound was isolated and identified as compound-5(Daucosterol).

Structural elucidation was established by using various modern spectroscopic techniques such as infrared(IR),nuclear magnetic resonance(NMR) including 1D-NMR(1H,13C) ,HRMS and Mass spectroscopy.The work on fruit yielded a total 5 compounds the structures of the compounds were also elucidated by comparing their physical properties and spectral data reported in the literature.

Further screening of biological activities like antioxidant activity of isolated compounds of hexane extract of fruits of *Aphanamixispolystachya* was done by $ABTS^{+}$ assay method. Scavenging of $ABTS^{+}$ Free Radical was performed as described by Walker and Everette with suitable modifications. Ascorbic acid was used as Standard .The compound 2(Aphanamixin-B) show moderate activity and the other compounds showed very less activity and compared with standard drug.

The α-Glycosidase Inhibition assay was performed as described by Babu*et al.* The compound 3 (Aphananin)showed good activity, compound 1(Stigmasterol)show moderate activity compared with standard drug. Acarbose is used as standard. Compound 2, 4 and 5 didn't showtheactivity.

The hexane extract of fruits of *Aphanamixispolystachya* was shown to be rich in phytochemical constituents and antioxidant and antidiabetic activity shown by the isolated compound 3 and compound 2 from the hexane extract of fruits of *Aphanamixispolystachya*. Further studies according to literature review, many pharmacologically significant chemical constituents and even new chemical constituents were isolated; therefore these remaining fractions can be

further necessary for the synthesis of the analogues of the most potent compounds based on the biological activity.

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