PRELIMINARY STUDIES ON EFFECT OF BIODIVERSITY ON ACTIVITY OF *POLYALTHIA LONGIFOLIA*

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Summary

Medicinal and aromatic plants represent a consistent part of the natural biodiversity endowment of many states in India, as well as the world at large. Medicinal and aromatic plant species are widely distributed due to a variety of climatic factors and altitudinal variations coupled with varied ecological habitats. These plant species are basic ingredient of the ethno-botanical and traditional health care system. The current study terminate the phytochemical analysis and antibacterial activity of ethyl acetate extract of different places collected dried leaves of *Polyalthia longifolia* against four different bacterial strains Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus. Comparison to different places collected species extract, Delhi collected *Polyalthia longifolia* extract shows maximum chemical constituent with significant antibacterial activity against the reference strain.

Key Words: - *Polyalthia longifolia*, Phytochemical Analysis, Antibacterial Activity.

Introduction

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolite substances which are in turn used to restore health and heal many diseases. Plants and plants products are being used as a source of medicine long. According to the world health organization more than 80% of the world population mostly in poor and less developed countries depends on traditional plant based medicine for their primary health care needs. Herbs are natural form of whole plants or their parts such as flower, root, oil, stem rich in bioactive chemical compounds so called "Herbiceuticals". The main difference between pharmaceutic drug and herbal principles is their isolation method and purification level. The pharmaceutical drugs are available with high purity as artificial chemical(s) while herbs are in rich natural complex chemicals. [1] Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional, Oriental and Indian medicine. Many drugs commonly used today are of herbal origin because of their safety, quality and efficacy. *Polyalthia longifolia* is native to the drier region of India and it is locally known as "Asaphala". The plant grows throughout the tropical and subtropical parts of India up to an altitude of I500 m. A tall, evergreen, handsome, pyramid like, columnar tree, undivided, growing up to 12m or more. Branches, Short, about 1-2 m long, glabrous and pendulous. Leaves are alternate, estipulate, distichous, mildly aromatic. Shining, glabrious, narrowly lanceolate. *Polyalthia* is a large genus of shrubs and trees distributed in tropic and subtropic regions. [2] It belongs to the family Annonaceae. Various parts of *P. longifolia* are used to treat fever, gonorrhea, uterus ailment, leucorrhoea and

menorrhagia. [3-6] Decoction made from bark is used as cure for mouth ulcers. [7] P. longifolia mainly contains diterpenoids, alkaloids, tannins, and mucilage. The chief components of the plant are O methylbulbocapnine-N-oxide, polyfothine, N-methylnandigerine-N-oxide, oliveroline-N-oxide, pendulamine A, N-pendulamine B, 8-oxopolyalthiane, 16-oxo-5 ,13-halimadien-15-oic acid, 16-Oxo-3, 13-clerodadien-15-oic acid, 16-hydroxycleroda-3, 13-dien-16, 15-olide. [8-13]

Materials & Methods

Collection and Authentication of Plant material:-

The leaves of *Polyalthia longifolia* were collected in Feb., 2009, from different places of India (Jaipur, Ahmadabad, Chandigarh and Delhi). The species was authenticated by Mr. Vinod Sharma, Herbarium Incharge, Dept. of Botany, Rajasthan University, Jaipur. A voucher specimen was deposited in the herbarium of the Dept. of Botany, Rajasthan University, Jaipur. The leave of plants were dried at room temperature separately and pulverized.

Extraction:-

Dried and powdered leaves of *Polyalthia longifolia* were washed separately with water and shade dried. The dried leaves were crushed to coarsely powdered by wood-grinder. The powdered material was extracted with ethyl acetate (60-80 ºC) in Soxhlet apparatus. The extract was separately concentrated for further studies on water bath at 40ºC. [14]

Microorganism:-

Four Reference Bacteria, Vic. Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATTCC 27853 and Bacillus cereus ATCC 6633 were used during the present study and were obtained from Dr. B. Lal Institute, Jaipur, Rajasthan. The bacteria were grown in nutrient broth at 37^0C and maintained on nutrient agar slants at 4^0C.

Phytochemical Analysis:-

Phytochemical screening was done for analyzing secondary metabolites that are responsible for curing ailments. The phytochemical screening of the plant extract was carried out in all different region collected leaves extracts.

Test for Alkaloids:

Dragendorff's test: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test: To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Hager's test: To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colored precipitate indicates the presence of alkaloids.

Wagner's test: To the 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins: Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. Layer of foam indicates the presence of Saponins.

Test for Glycosides:

Legal's test: Dissolve the extract in pyridine and add sodium nitropruside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

Baljet's test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

Keller-Killani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate

and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of
2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of Anthraquinone glycosides.

Test for Carbohydrates:

Molisch's test: To 2ml of the extract, add 1ml of a-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

Test for Tannins:

i)Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

ii) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins. The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

iii) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and Phenolic compounds.

Test for Flavonoids:

Shinoda's Test: i) The extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols.

ii) The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

iii) The extract is treated with concentrated H2SO4, formation of yellow or orange color indicates flavones.

Test for Steroids:

Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H2SO4. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract. [14]

Test for Fats & Oils:

i) Place a thick section of drug on glass slide. Add a drop of Sudan Red III reagent. After 2 min. wash with 50% alcohol. Mount in glycerin. Observe under microscope. Oil globules appear red.

ii) Place little amount of drug sample on the filter paper and stand for 15 minutes. A greasy spot observe due to presence of fats. [15]

Antibacterial Assay:-

Muller- Hinton Agar plates are prepared by pouring 10-15 ml of the medium in to each sterilized petridis and are allowed to set at room temperature. The cell suspension is inoculated over the surface of agar medium using sterile cotton swab. The two cups are scooped in each plate using a sterile cork borer of 8 mm diameter. Then the solution of test

compounds (100 µl and control 100 µl) are added in cups by using micropipettes and these plates are incubated at 37^0C for 48 hr. Standard drug ciprofloxacin (5 mcg) is used. The Zone of inhibition is measured in mm for each organism. [16]

Results

Phytochemical Analysis:

The different region collected *Polyalthia longifolia* leaves ethyl acetate extract are subjected to Phytochemical screening to detecting nature of Phytoconstituents. Ethyl acetate extract of *Polyalthia longifolia* shows the presence of carbohydrates, glycosides, tannins, Flavonoids & steroids mainly. From all four region collected samples, Delhi region collected samples contains maximum constituent which is shown in Table no. 1.

S. No.	Phytoconstituents	Leaves Extract Jaipur Region	Leaves Extract Ahmadabad Region	Leaves Extract Chandigarh Region	Leaves Extract Delhi Region
1	**Carbohydrates**	Positive	Positive	Positive	Positive
2	**Amino acids**	Positive	Positive	Positive	Positive
3	**Glycosides**	Positive	Positive	Positive	Positive
4	**Alkaloids**	Negative	Negative	Negative	Negative
5	**Flavonoids**	Positive	Positive	Positive	Positive
6	**Tannins**	Positive	Positive	Positive	Positive
7	**Saponins**	Negative	Negative	Negative	Positive
8	**Steroids**	Positive	Positive	Positive	Positive
9	**Oils & Fats**	Positive	Positive	Positive	Positive

Table -1 – Phytochemical Screening of different places collected leaves extract of *Polyalthia longifolia*.

Antibacterial Assay:-

Four different collected *Polyalthia longifolia* extract is test for antibacterial activity. The doses of 1000 mg/ml of extracts were made by dissolving appropriate quantity of extracts in DMSO. The solutions of test compounds (100 µl and control100 µl) are added in cups by using micropipettes and these plates are incubated at 37^0C for 48 hr. The Zone of inhibition is measured in mm for each organism. Controls with DMSO did not show any activity. The different crude extracts show positive antimicrobial activity against both gram positive and gram negative bacteria. The results shown in Table No. 2.

S.No.	Drug → / Microorganism ↓	Leaves Extract Jaipur Region	Leaves Extract Ahmadabad Region	Leaves Extract Chandigarh Region	Leaves Extract Delhi Region	Ciprofloxacin
1	E. coli	8 mm	7mm	9mm	10mm	43 mm
2	S. aureus	21mm	19mm	18mm	26mm	32mm
3	P. aeruginosa	26mm	28mm	25mm	29mm	39mm
4	B. cereus	29mm	31mm	28mm	35mm	38mm

Table -2 – Showing effect of different places collected leaves extract of *Polyalthia longifolia*.

Discussion

The Phytochemical study of *Polyalthia longifolia* leaves shows plant mainly contains tannins, phenolic acids, glycosides and steroids. Presence of flavonoids and tannins in *Polyalthia longifolia* responsible for antibacterial activity. [17] In this present study different places collected *P. longifolia* species showing variation in chemical constituent due to biodiversity. Comparison to different places species extract, Delhi collected *Polyalthia longifolia* extract shows significant antibacterial activity against the reference strain.

Conclusion

Medicinal and aromatic plant species are widely distributed due to a variety of climatic factors and altitudinal variations coupled with varied ecological habitats. On basis of results it may be concluded that plants grown in different biosphere may have variation in their chemical constituent as well as activity, as was observed in case of *Polyalthia longifolia*, collected from various places. However, further detailed studies are needed for establishing this fact.

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