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Evaluation of Genoprotective Activity of *Indigofera tinctoria* using *Allium cepa* Root

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The principle reason for the examination article is to assess the genoprotective action of the herbal source leaves extract of *Indigofera tinctoria* against insecticidal such as transfluthrin using *Allium cepa* as a biomarker. *Indigofera tinctoria* has the potential secondary metabolites, inorganic elements in phytochemical screening and different functional groups also. So that It possesses excellent anticancer, anti-inflammatory, anti-oxidant, antifungal, antibacterial, antitumor, anti mycobacterial, mosquitocidal, immunostimulatory properties. Normally for mutation, chemotherapy and radiation treatments are used. Here we used herbal remedy as *Indigofera tinctoria* against transfluthrin by using *Allium cepa* as a biomarker and the mitotic record and DNA discontinuity likewise determined. It contains high amount of secondary metabolites, inorganic elements and functional groups. The mitotic index remains exactly same at before and after treatment by calculating the number of roots and its length in segments like dividing and non-dividing cells. We concluded that *Indigofera tinctoria* repaired the mutation act against insecticidal transfluthrin. So it acts as an excellent mutant repair mechanism against insecticidal.

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Keywords: Transfluthrin; biomarker; Indigofera tinctoria; Allium cepa; mitotic index.

1. INTRODUCTION

Toxins are only the substance that can hurt people or creatures. In interrelated genotoxicity alludes to the character of artificial compounds relating their capacity to make injure to hereditary material. The word *mutagen* refers to a substance that induces infectious changes in DNA structure connecting a group of genes or single gene. In hereditary qualities, genotoxicity is a term that depicts the property of artificial compounds relating to their capacity to make harm hereditary material [1]. Genotoxicity can prompt circuitous or direct consequences for the DNA including change enlistment, confused occasion initiation, and direct DNA harm which prompts transformations. It causes immediate, heritable changes that would be passed to people in the upcoming of cells [2]. Genotoxins contains chemical elements and radiation. Estimation of the genotoxicity effects of a diversity of materials for employ as drug mechanism is necessary. Approximately partially of all Pyrrolizidine alkaloids is classified as genotoxic and lots of are tumorigenic. A variety of chemicals have the probable to be genotoxic, and by addition, mutagenic. Beyond few techniques and methods can be accessible for genotoxicity testing. A leaf mixture of *Indigofera tinctoria* (from time to time combined with honey or milk) is worn to care for a scope of confusion checking epilepsy and apprehensive issues; grievance of the stomach, fever; and spleen; liver, kidney, asthma and bronchitis; and as a rabies prophylactic. Reasonable ostensibly, the leaves are made into a cream for treating skin infections, injuries, ulcers, wounds and hemorrhoids. A slim root glue is utilitarian topically in India to really focus on worm-invaded wounds. A root mix is worn there as a remedy contrary to snakebites and to treat bug and scorpion stings. Onion a potential biomarker of genotoxic examines. Extensively used as a bio-indicator of genotoxicity from the dissimilar aquatic vicinity. This test used to assess mutagens and detect poisonous substances found in surroundings. Plant roots are very useful in biological testing since root tips are the initial to be exposed to toxicants discrete in soil or in water. Therefore, the root tip chromosomal deviation assays comprise rapid and responsive methods for biomonitoring the level of pollution and to assess the effects of toxic and mutagenic substances in the usual surroundings [3]. It is a type of insecticide. It comes under the group of pyrethroid. It has a substance origin of synthetic. Normally genoprotectivity damages are bridge formation, stickiness of chromosomes at prophase and metaphase ranges, disturbance inside the orientation of the spindle resulting in wrong guidelines of chromosomes, were the common abnormalities located within the case [4]. *Indigofera tinctoria* more desirable innate in addition to adaptive immune reaction and proved the immunostimulating ability. The present study was performed to evaluate genoprotective activity of *Indigofera tinctoria* using *Allium cepa* as a biomarker and which utilized in repair the mutation against the insecticidal transfluthrin and various properties like as antifungal, antibacterial, antitumor, antimycobacterial, mosquitocidal and immunostimulatory.

2. MATERIALS AND METHODS

2.1 Determination of *Indigofera tinctoria* Alcohol Soluble Extract

Precisely gauged test leaves powdered material (4 g) was put in a glass stoppered round base carafe (RBF). Ethanol (100 ml) was added to the RBF and afterward, it was shaken well and permitted to represent 1hr. A reflux condenser was appended and bubbled tenderly for 1h and afterward it was cooled and separated. The cup was shaken well and separated quickly through a dry channel paper. From that point onward, 25 ml of the filtrate was moved to a tarred level lined dish and vanished to dryness on a water shower. At that point the dish was dried at 105 °C for 6 hour and cooled in a desiccator and gauged. 0.1% ferric chloride is added and observed for brownish green or a blue-black colouration [5].

2.1.1 Test for Saponin

About 2 ml of test is bubbled in 20 ml of refined water in a water shower and separated. 10 ml of the filtrate is blended in with 5 ml of refined water and shaken energetically for stable diligent foam. The foaming is blended in with 3 drops of olive oil and shaken properly, at that point noticed for the positioning of emulsion.

2.1.2 Test for Flavonoids

5 ml of weaken smelling salts arrangement were added to a segment of the watery filtrate of each

plant separate followed by option of concentrated H_2S0_4. A yellow colouration saw in each concentrate demonstrated the presence of flavonoids. The yellow colouration vanished on standing.

2.1.3 Test for Steroids

2 ml of acidic anhydride is added to 1ml of concentrate of each example with 2 ml H_2SO_4. The concealing changed from violet to blue or green in specific models showing the presence of steroids.

2.1.4 Test for Terpenoids (Salkowski test)

5 ml of each move is mixed in 2 ml of chloroform, and thought H_2SO_4 (3 ml) is intentionally added to outline a layer. A ruddy earthy colored colouration of the interface is framed to show positive outcomes for the presence of terpenoids.

2.1.5 Test for Triterpenoids

1 ml of the concentrate is included 1 ml of chloroform; 1 ml of acidic anhydride is added following the option of 2 ml of concentrated H_2SO_4. Arrangement of ruddy violet tone demonstrates the presence of triterpenoids.

2.1.6 Test for Alkaloids

2.1.6.1 Mayer's test

To a couple (one) ml of the concentrate, a drop of Mayer's reagent is added by the side of the test tube. A velvety or white encourage demonstrates the test is positive.

2.1.6.2 Test for Coumarins

2 ml of each extracts is treated with 3 ml of 10% NaOH. A yellow colouration observed in each extract indicated the presence of coumarins.

2.2 Quantitative Analysis of Phytochemicals

2.2.1 Determination of total phenols by spectrophotometric method

Plant powder (2g) was overflowed with 50 ml of ether for the extraction of the phenolic part for 15 min. 5 ml of the concentrate was pipetted into a 50 ml flask, then, at that point 10 ml of distilled water was added [6]. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl liquor were likewise added. The examples were made up to stamp and left to respond for 30 min for shading event. It was estimated at 505 nm.

2.2.2 Determination of Flavonoids

10 g of the plant test was removed over and over with 100 ml of 80% watery methanol at room temperature. The entire arrangement was separated through Whatman channel paper No 42 (125 mm). The filtrate was subsequently moved into a cauldron and dissipated into dryness over a water shower and weighed to a steady weight [7].

2.2.3 Estimation of total terpenoid content

1 g of plant powder was taken independently and absorbed liquor (50ml) for 24 hrs. At that point separated, the filtrate was extricated with petrol ether (40ml) for 2 hours. The dried ether separate was dissipated by complete disposal of petrol ether under diminished tension. The dried ether separate was treated as all out terpenoid [8].

2.3 Determination of Fluorescence Behavior of Plant Powder

Fluorescence examination of seeds powder of *Indigofera tinctoria* leaves has been completed in sunlight and under UV light [9]. Brilliance examination of seeds powder of *Indigofera tinctoria* leaves were done by the treatment of various substance reagents like $AlCl_3$, H_2SO_4, HCl, NH_3, CH_3OH, HNO_3 and NaOH. The powders were seen in typical sunshine and under short (245 nm) and long UV light (365 nm) [10].

2.4 Qualitative Analysis of Inorganic Elements

Plant powder (500mg) was arranged and treated with HNO_3 and HCl (3:1 v/v) for 60 minutes. After the filtration, the filtrate was utilized to play out the accompanying tests [11].

2.4.1 Calcium

One drop of dil. ammonium hydroxide and splashed ammonium oxalate game plan was added to 10ml of the above filtrate. White accelerates of calcium oxalate, dissolvable in hydrochloric acid yet insoluble in acidic acid, were shaped.

2.4.2 Magnesium

White calcium oxalate encourage was isolated by sifting the above arrangement. The filtrate was warmed and cooled. Arrangement of sodium phosphate in weaken alkali arrangement was added. White glasslike encourage was noticed.

2.4.3 Sodium

Little uranyl magnesium acidic corrosive inference reagent was added to 2ml of the test course of action, shaken well and put something aside for few seconds. Yellow translucent encourage of sodium magnesium uranyl acetic acid derivation was noticed.

2.4.4 Potassium

Not many drops sodium cobalt nitrite arrangement was added to 2-3ml of the test arrangement. Yellow accelerate of potassium cobalt nitrite was noticed.

2.4.5 Iron

Scarcely any drops of 2% potassium ferrocyanide were added to 5ml of the test arrangement. Dull blue tinge was noticed.

2.4.6 Sulphate

To 5ml of the test arrangement, lead acetic acid derivation reagent was added. A white hasten, solvent in sodium hydroxide, was framed.

2.4.7 Phosphate

5ml of test arrangement was set up in nitric acid and a couple of drops of ammonium molybdate arrangement were added. It was warmed for around 10 minutes and left to be cooled. A yellow glasslike hasten of ammonium molybdate was noticed.

2.4.8 Chloride

3 to 5ml of lead acetic acid derivation arrangement was added to around 5 to 7ml of the filtrate. A white accelerate solvent in steaming hot water was noticed.

2.4.9 Nitrates

Ferrous sulfate arrangement was added to 5ml of the test arrangement. No earthy colored tone was delivered, yet when sulphuric acid was added (gradually from the side of the test tube), an earthy colored shaded ring was created at the intersection of two fluids.

2.5 Qualitative Analysis of Vitamins

2.5.1 Vitamin- A

Break down 1 ml of test in 5 ml of chloroform and separated. Add 5ml of antimony trichloride arrangement. A transient blue tone is created right away.

2.5.2 Vitamin-C

Weaken 1 ml of watery *Indigofera tinctoria* arrangement with 5 ml of water and add 1drop of newly arranged 5% w/v arrangement of sodium nitroprusside and 2 ml of weaken sodium hydroxide arrangement. Add 0.6 ml of HCl drop savvy and mix, the yellow shading becomes blue.

2.5.3 Vitamin –D

Disintegrate a 1 ml of test in 10 ml of chloroform and separated. Add 10ml of antimony trichloride arrangement. A pinkish - red tone shows up immediately.

2.5.4 Vitamin -E

1 ml of the *Indigofera tinctoria*was macerated with 10ml of ethanol for 5 minutes and afterward sifted. Barely any drops of 0.1% ferric chloride in ethanol and 1ml of 0.25% of 2'- 2'dipyridyl to 1ml of the filtrate. Brilliant red tone was framed on a white foundation. The foundation steadily expects to be a pink [12].

2.6 FTIR Spectroscopic Analysis

The methanol extricate were analyzed under FTIR spectrophotometer examination were filtered in the frequency going, trademark tops were identified [13].

2.7 Column Chromatography Analysis

2.7.1 Extraction of polyphenolic compounds

Extraction of phenolic compounds along with sugars was carried out in the following manner. Twenty five grams of sample (Powder) were introduced into a 100 ml dark glass bottle and suspended in 200 ml of methanol-water or

acetone-water (80:20, v/v). Tightly capped bottles placed in water bath at 80 ^{0}C. After 15 min during which the content was shaken twice, the extract was cooled and filtered under partial vacuum. The material left on the filter paper was transferred back to dark glass bottles for further extraction with 200 ml of the same extraction solution [14]. This methodology was rehashed multiple times more than 30 and 60 min of extraction, each time gathering the answer for analysis. Supernatants were joined and dissipated utilizing rotating vacuum evaporator to eliminate any leftover dissolvable; the water was then taken out by lyophilization [15].

2.7.2 Column Chromatography

The column of about 15 cm long and 4 cm in width was completely washed with cleanser, flushed with refined water and afterward permitted to dry. At the point when the column has been completely dried, little piece of glass fleece was embedded into the lower part of the column and the column was upheld using a clap and report stand. A channel is connected to the open end and minimal clean white sand was poured on top of the glass fleece previously embedded, after this some amount of the dissolvable was poured down the column. The silica gel (40 micron) utilized was initiated in the broiler at 120 ^{o}C f or 2 hours. An all around mixed suspension of silica gel (100–150 g in oil ether at 60 °C – 80 °C was filled a column (150 cm long and 50 mm in width). At the point when the spongy was very much settled, the abundance of petroleum ether was permitted to go through the column. The slurry was gone through the silica gel in petroleum ether and was processed to very much blended column. Slow setting was orchestrated by keeping a delicate disturbance while there was dissolvable move through the column to acquire a homogenous pressing [16]. A 1.0 g part of the concentrate was broken down in 5 ml of methanol and applied to a chromatographic column (3.4 x 50 cm) loaded with Sephadex LH-20 and eluted with methanol. Portions (6 ml) were gathered utilizing a division authority and their subjective examination of polyphenol test [17].

2.7.2.1 Polyphenol test

Ethanol (4 ml) is added to each fraction (1ml) and the ensuing plan is moved in test tubes and warmed in a water shower (15 minutes). Three drops of recently organized ferric cyanide game plan were added to the concentrate course of action. Improvement of a blue green concealing showed the presence of polyphenol.

2.7.3 Thin layer chromatography

A thin-layered plate is set up by spreading fluid slurry of Silica gel G on the spotless surface of a glass or unbending plastic. Calcium carbonate or starch is likewise added to the adsorbent to expand grip. The plate is then warmed in a broiler for around 30 mins at 105 °C to actuate the plate. It is then cooled inside the actual stove. Test tests were applied as spots utilizing slim cylinder [18]. The selection of solvents relies on the idea of compound to be isolated and furthermore on the adsorbent utilized. The dissolvable is filled the chamber and shut firmly and the chamber is soaked for a couple of hours prior to running the chromatogram. The eluted portions were described through thin layer chromatography on silica gel plates utilizing water-methanol-chloroform (10:35:65, v/v/v) as the creating framework followed by showered with a watery arrangement of ferric chloride to imagine phenolic compounds [19,20].

R_f Value

It is a proportion of distance went by the example and distance went by the dissolvable.

$$R_f = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

2.8 Evaluation of Genoprotective Activity using *Allium cepa* Root

Genoprotective action study was led according to the strategies announced by past laborers with adjustments.

2.8.1 *Allium cepa* Bulbs

Genoprotective action study was led according to the strategies announced by past laborers with adjustments.

2.8.2 Growing *Allium cepa* Meristems

The external scales were eliminated from the sound onion bulbs leaving the root primordia unblemished. These bulbs were filled in dim for 48 h more than 100 ml of faucet water at surrounding temperature until the roots have developed to around 3 cm. The water was

changed every day during this period. The practical bulbs were then chosen and utilized for resulting contemplates [21,22,23].

2.8.3 Exposure to Test Samples

The bulbs with root tips grown up to 2-3 cm were eliminated from the water and put on a layer of tissue paper to eliminate overabundance of water. The bulbs were partitioned into four gatherings. The primary gathering filled in as control (faucet water). Second gathering got just Transfluthrin (100 µg/ml). Third gathering: *Allium cepa* establishes were plunged in the Transfluthrin (100 µg/ml) + *Indigofera tinctoria* leaves extricate (100 µg/ml). Fourth gathering: *Allium cepa* establishes was plunged in the Transfluthrin (100µg/ml) + *Indigofera tinctoria* leaves separate (200 µg/ml). Fifth gathering: *Allium cepa* establishes was plunged in the Transfluthrin (100 µg/ml) + *Indigofera tinctoria* leaves separate (400 µg/ml). Every one of the gatherings was brooded at 25±2°C for 72 h away from direct daylight. The test tests were changed every day with new ones. The length of roots developed during hatching (recently showing up roots excluded), root number and the mitotic list were recorded after 72 h [24,25].

2.8.4 Microscopic Studies and Determination of Mitotic Index

After 96 h, the root tips were fixed with fixing arrangement of acidic acid and liquor (1:3). Squash arrangements were made by staining the treated roots with acetocarmine stain. For each root tip, the quantities of mitotic cells and all out meristematic cells were included physically in 5-8 fields of view utilizing high goal (100 x) brilliant field light microscopy. The mitotic list was established as :

Mitotic Index = Number of dividing cells/Total number of cells x 100 [26,27].

2.8.5 Deoxy Ribonucleic Acid (DNA)

A known volume (1.5 ml) of the nucleic acid concentrate was made upto 3.0 ml with 1N perchloric acid. This was blended in with 2.0 ml of diphenylamine reagent. A reagent clear and norms were likewise done simultaneously. This was kept in a bubbling water shower for 10minutes and the blue shading created was perused at 640 nm in a spectrophotometer [28]. The DNA level was expressed as mg/g wet sample.

2.9 Analysis of DNA Fragmentation

Tissues were homogenized with four volume of lysis cradle and centrifuged at 16, 000 rpm for 20 min to isolate flawless DNA from divided DNA. After move of the supernatant to a different cylinder, 0.2 ml of lysis cradle was added to the pellet. To both divided and unblemished DNA portions, 0.2 ml of perchloric acid was added. In the wake of keeping up the blend at 4 ^{o}C for 30 min, the examples were centrifuged at 16,000 rpm for 20 mi advertisement the supernatant was eliminated. The pellets were disintegrated in 0.05 ml of perchloric acid and warmed at 70^{o}C for 20 min. At that point 0.1ml of diphenylamine reagent was added and the shading created was perused at 600nm [9].

2.10 Statistical Analysis

The outcomes were introduced as Mean ± SD. Information were investigated by single direction ANOVA followed by post-hoc Tukey HSD test, information were measurably critical level alpha 0.05 utilizing SPSS version 20.

3. RESULT AND DISCUSSION

3.1 Extractive Value Analysis

Alcohol and water soluble extract value of *Indigofera tinctoria* leaves investigated. Among the two extract value, alcohol extract (24.80%) value has higher than water extract (12.80%) value [29].

3.2 Qualitative and Quantitative Anlaysis

Qualitative and quantitative analysis of *Indigofera tinctoria* leaves studied (Table 1). Significant amount of flavonoids (90.00±6.30), terpenoids (40.00±2.80), and phenol (152.38±10.66) were present in *Indigofera tinctoria* leaves. The above phytoconstituents were tested as per the standard methods. Values are expressed as mean ± SD for triplicates (Fig. 1).

3.3 Minerals and Vitamins Analysis of *Indigofera tinctoria* Leaves

The Minerals of *Indigofera tinctoria* leaves showed that the presence of Calcium, Magnesium, Sodium, Potassium, and sulphate, chloride, Phosphate and nitrate while iron was

absent. The current examination showed the presence of Vitamin C and Vitamin E while Vitamin D and Vitamin A was absent in *Indigofera tinctoria* leaves [30].

3.4 Fluorescence Behavior of *indigofera tinctoria* Leaves Powder

Fluroscence examination of entire leaves of *Indigofera tinctoria* has been done in daylight and under uv light. Fluroscence assessment of leaves powder of *Indigofera tinctoria* was done by the treatment of different chemical reagents [31,32].

3.5 FTIR Analysis of *Indigofera tinctoria* Leaves Extract

FTIR also refered as FTIR spectroscopy is an FTIR evaluation is an analytical move toward used to perceive organic, polymeric and in some cases, inorganic substances. The FTIR analysis technique makes use of infrared mild to experiment take a look at samples and examine chemical residences [33].

3.6 Column Chromatography

The separation and acknowledgment of the phenolic compound utilizing Column chromatography and TLC from *Indigofera tinctoria* leaves. The *Indigofera tinctoria* leaves removed with methanol-water (80:20, v/v).This methodology was rehashed multiple times more than 30 and 60 min of extraction. The most noteworthy extraction of phenolic compounds from plant material with methanol-water (80:20, v/v) was accomplished during the extraction. The gathered parts additionally tried in phenol by subjectively. Every one of the parts showed the solid positive response in phenol and concentrated the divisions for additional TLC examination. Attention figure showed the existence of Gallic acid and quercetin as contrasted and the standard Gallic acid and quercetin. The result of the investigation reasoned that *Indigofera tinctoria* leaves separate contain Gallic acid and quercetin [34].

3.7 Genoprotective Activity of *Indigofera tinctoria* Leaves Extract

In *Allium cepa* L. root tip model root arrangement of plant cells is ordinarily utilized as a test for researching ecological contamination factors, Poisonousness of substance compounds and Assessing likely anticancer properties. The point of this work was research the Genoprotective action of *Indigofera tinctoria* leaves.

Values were expressed as mean ± SD for triplicate in each group as mitotic slide cell counting. Data were analyzed by one-way ANOVA followed by post-hoc Tukey HSD test. Statistically significant variation was derived by comparing Group I versus Group II, Group III, Group IV and Group V. Significance level α 0.05.*P<0.05 statistically significant and NS= Non significant (P>0.05) compared with Group I (Normal).Overall effect on *Indigofera tinctoria* leaves extract were Group II, Group III and Group IV was statistically significance deference from α 0.05 significant level and Group V was Non-significant from α 0.05 significant level compared with Group I.

Table 1.Analysis of *Indigofera tinctoria* leaves

S. No	Test	Visible Light	Short UV Light (254nm)	Long UV Light (365nm)
1	Plant powder	Light green	Light green	Greenish dark black
2	Plant powder treated with distilled water	Dark green	Light green	Greenish dark black
3	Plant powder treated with Hexane	Light green	Yellowish green	Greenish dark black
4	Plant powder treated with Chloroform	Dark green	Dark green	Greenish dark black
5	Plant powder treated with Methanol	Light green	Yellowish green	Greenish dark black
6	Plant powder treated with Acetone	Dark green	Dark green	Greenish dark black
7	Plant powder treated	Dark green	Light green	Greenish dark

S. No	Test	Visible Light	Short UV Light (254nm)	Long UV Light (365nm)
	with 1N Sodium Hydroxide			black
8	Plant powder treated with 1N Hcl	Dark green	Dark green	Greenish dark black
9	Plant powder treated with sulphuric acid with equal volume of water	Dark green	Dark green	Greenish dark black
10	Plant powder treated with Nitric acid diluted with an equal volume of water	Reddish brown	Pale green	Greenish dark black

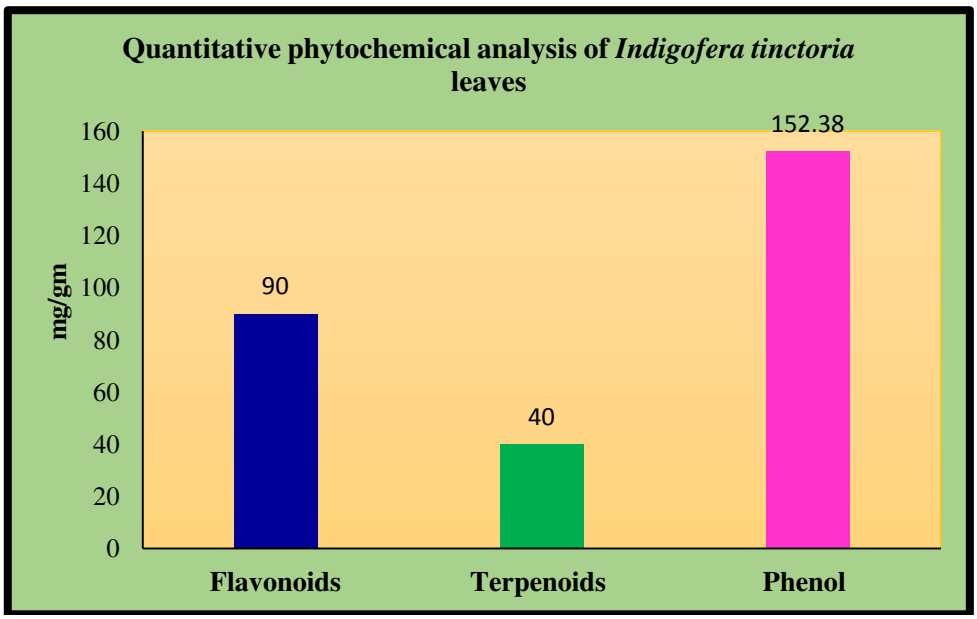


Fig. 1. Quantitative phytochemical analysis of *Indigofera tinctoria* leaves powder

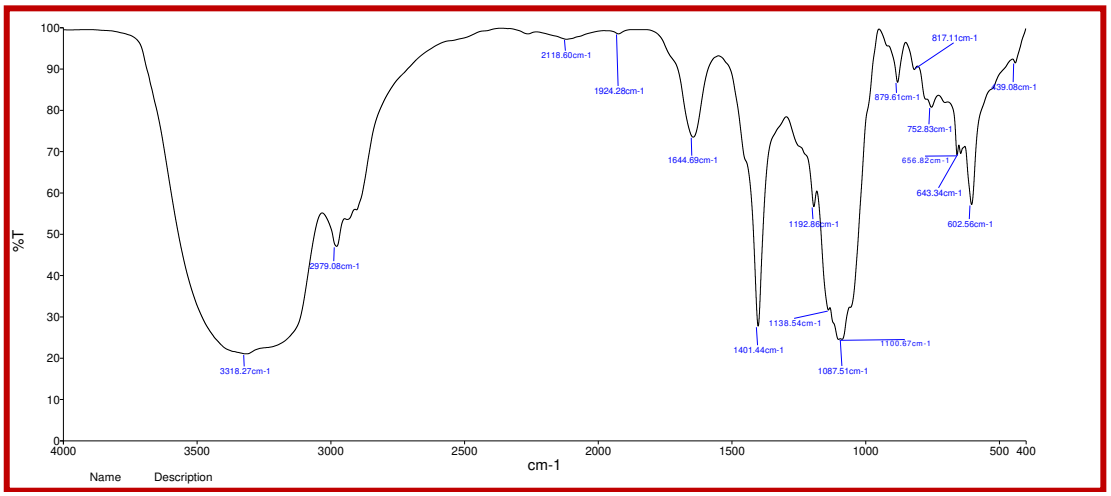


Fig.2. FTIR peak of *Indigofera tinctoria*

Table 2. FTIR peak values of *Indigofera tinctoria*

Frequency(cm^{-1})	Bonds	Functional groups
3318.27	O–H stretch, H–bonded	Alcohols, Phenols
2979.08	O–H stretch	Carboxylic acids
2118.60	–C ≡C– stretch	Alkynes
1644.69	–C=C– stretch	Alkenes
1401.44	C–C stretch (in–ring)	Aromatics
1087.51	C–N stretch	Aliphatic amines
752.83	C–Cl stretch	Alkyl halides

Table 3. Separation and identification of the phenolic compound using Column chromatography and TLC from *Indigofera tinctoria* leaves extract

Fraction	Fraction colour	Qualitative analysis polyphenol	R_f value
1	Yellow	+	0.93
2	Brown	+	0.82
3	Green	++	0.65
Standard (Gallic acid)			0.61
Standard (Quercetin)			0.68

(+) presence; (++) present with high intensity of three colour



Fig. 3. Identification of the phenolic compound using Column chromatography eluting and TLC from *Indigofera tinctoria* leaves extract



Fig. 4. Genoprotective activity of *Indigofera tinctoria* leaves extract in experimental step up

Table 4. Effect of *Indigofera tinctoria* leaves mitotic index of *Allium cepa* roots

Groups	Dividing Cells	Non Dividing Cells	Total cells	Mitotic Index (%)
Group I (Control)	110±7	15±3	125±10	88.07±1.45
Group II (Transfluthrin only)	21±5	68±2	89±3	23.50±4.41*
Group III (Transfluthrin+ 100µg/ml)	51±3	54±3	105±2	48.26±2.42*
Group IV (Transfluthrin + 200µg/ml)	61±4	33±3	94±7	65.06±1.73*
Group V (Transfluthrin + 400µg/ml)	98±7	12±3	111±8	88.58±2.08^{NS}

Table 5. Effect of *Indigofera tinctoria* leaves root number and root length of *Allium cepa* roots

Groups	Root Number and length (cm)			
	Before treatment		After treatment	
	Number	length (cm)	Number	length (cm)
Group I (Control)	23	3.84±2.32	29	4.59±2.50
Group II (Transfluthrin only)	9	2.48±1.46	13	2.53±1.32
Group III (Transfluthrin + 100µg/ml)	7	4.50±1.57	9	4.17±2.14
Group IV (Transfluthrin + 200µg/ml)	3	7.30±0.66	5	8.33±1.06
Group V (Transfluthrin + 400µg/ml)	7	5.22±2.97	9	5.12±2.86

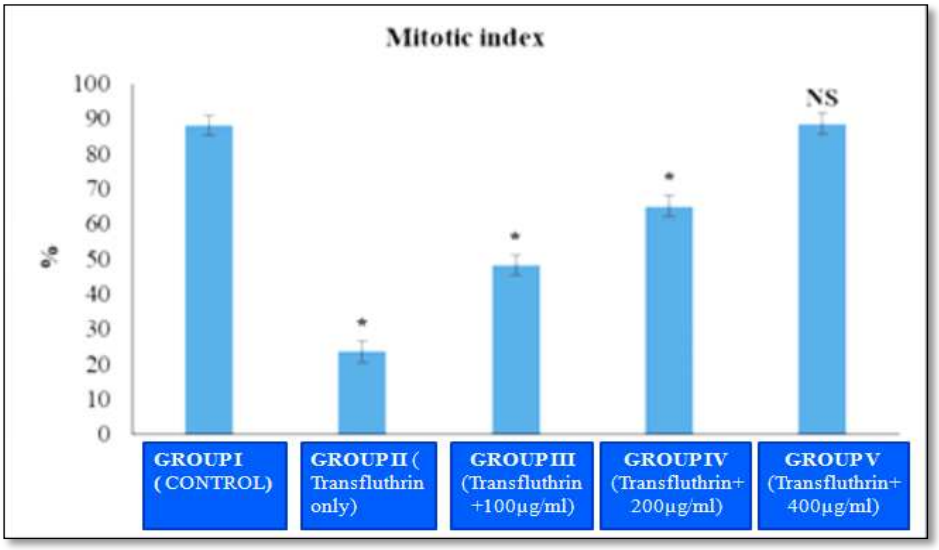
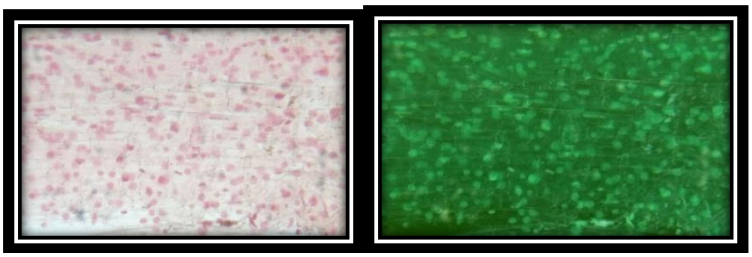


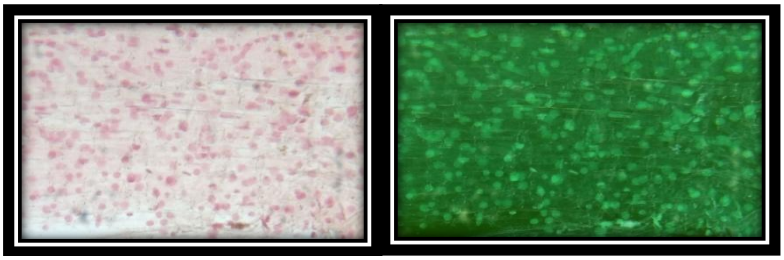
Fig. 5. Effect of *Indigofera tinctoria* leaves on mitotic index of *Allium cepa* root



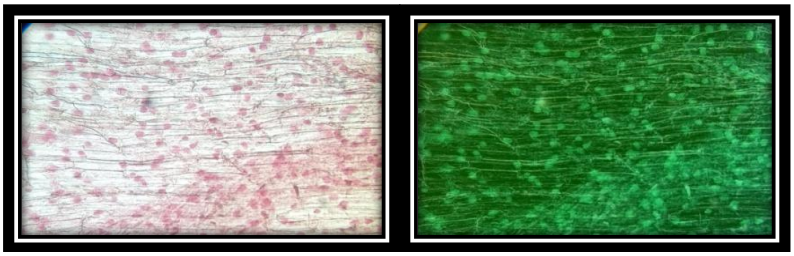
Group I (Control)



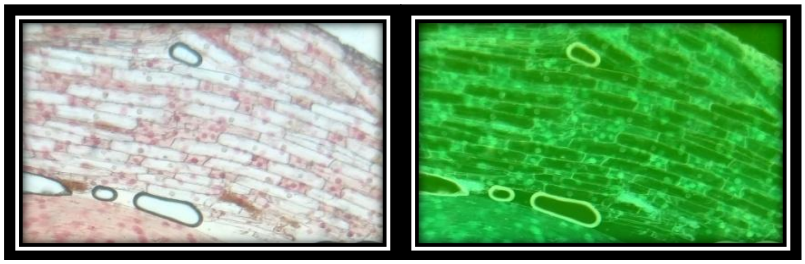
Group II (Transfluthrin only)



Group III (Transfluthrin + 100µg/ml)



Group IV (Transfluthrin + 200µg/ml)



Group V (Tranfluthrin + 400µg/ml)

Fig. 6. Effect of *Indigofera tinctoria* leaves on *Allium cepa* root

Table 6.Effect of *Indigofera tinctoria* leaves on DNA and DNA fragmentation in *Allium cepa* roots

Parameters	Group I	Group II	Group III	Group IV	Group V
DNA (mg/dl)	4.95 ± 0.17	1.81 ± 0.13*	2.37 ± 0.19*	3.18 ± 0.15*	4.37 ± 0.11^{NS}
DNA fragmentation (%)	6.03 ± 3.01	88.41 ± 3.34*	76.50 ± 6.09*	47.30 ± 3.57*	6.82 ± 2.57^{NS}

4. DISCUSSION

Phytochemical concentrates on the *Indigofera tinctoria* plants proved that it containing compounds like terpenoids, flavonoids, lignins, Nitro groups containing mixtures, steroids and concluded that *Indigofera tinctoria* showed against hyperglycemic, antibacterial, cytotoxicity, antidiabetic, hostile to inflammatory, against epilective, against hepatoprotective, antihelmenthic, antinoceptive, antiproliferative andantidyslipidemic activites. During genotoxicity effects like strand break, structural and/or numerical chromosomal aberrations, point mutations, cross-linking and loss of excision repair) in cells are happened and also during calculating mitotic index these are also included. Most of the functional group like epoxide, aziridine, alkyl sulfonates are causing genotoxicity. Similar to this observation, the FTIR spectrum was used to classify the functional group of the active components [35].

Onion (*Allium cepa* L.) a possible biomarker of genotoxic studies and extensively used as a bio-indicator of genotoxicity from the dissimilar aquatic vicinity. This test used to assess mutagens and detect poisonous substances found in the surroundings. It roots are very useful in biological testing since root tips are the initial to be exposed to toxicants discrete in soil or in water. Therefore, the root tip chromosomal deviation assays comprise rapid and responsive methods for biomonitoring the level of pollution and to assess the effects of toxic and mutagenic substances in the usual surroundings. Transfluthrin effects insects presynaptic voltage gate sodium channels in nerve membranes rapid causing knockdown. It has the ability to cause DNA damage rapidly in humans and animals also. Column chromatography is one of the most commonly employed separation methods to classify both organic and inorganic products, implying its possible utility in chemical analysis of complex extract content in this research.

The effectiveness of column-chromatographic techniques for the separation of biologically active secondary metabolites from plant samples was demonstrated in this study. The existence of complex potent bio molecules in this plant is confirmed by TLC profiling of all three extracts in Chloroform: Methanol .By this study, it was concluded that methanolic extract of (*Indigofera tinctoria*) analysis such as FTIR, Column chromatography and TLC were showed high activity compared to another plants. Exactly the same mitotic index (88.07) obtained from final value as initial value. From this concluded that Indigofera tinctoria act as excellent mutant repair capability against insecticidal mutation [36].

Various investigations suggest that performed the genotoxicity mitotic index (MI) and percent chromosomal abnormality (% CA) of the unrefined ethanolic concentrate product utilizing *Allium cepa* (onion) assay and they removed the plant parts and performed Onion Genotoxicity Assay for Chromosome Abnormality Screening .In contrast no information is available about *Indigofera tinctoria* acting as a mutation repairing agent and got exact mitotic index value.Many of the reports availble that *Indigofera tinctoria* have dye producing capability and antioxidant property [37]. Since we revealed that it remains same mitotic index by *Allium cepa* assay.we done the measurement of genotoxicity by considering how much amount of insecticidal dose exposure given,effect of insecticidal by oxidative stress assays, susceptibility of biomarker and amount of DNA damage [38 - 40].

5. CONCLUSION

The phytochemical screening *Indigofera tinctoria* leaves extract was choose based on high concentration of phytochemicals. The phytochemical screening *Indigofera tinctoria* leaves extract showed that the tannin, saponin, flavonoids, steroids, terpenoids, triterpeniods, polyphenol, glycosides and coumarins were present in aqueous extract. And tannin, saponin, flavonoids, steroids, terpenoids, triterpeniods, polyphenol, glycosides, antroquinone and coumarins were present in the alcoholic extract

such as ethanol. The quantitative significant amount of flavonoids, terpenoids and total phenol contained in *Indigofera tinctoria* leaves extract. The inorganic elements such as vitamin C and vitamin Existence in *Indigofera tinctoria* leaves extract confirmed by vitamin analysis. Also the Calcium, Magnesium, Sodium, Sulphate, Phosphorus, Nitrate, Chloride are present in *Indigofera tinctoria* leaves that confirmed by minerals analysis. Present examination was distinguished the Quercetin and gallic corrosive compound utilizing Column chromatography and TLC by got three distinctive shading portions. The FTIR investigation affirmed that Alcohols, Phenols,Carboxylic acids, Alkenes, Alkynes, Aromatics, Aliphatic amines, Alkyl halides present. The concentrate of *Indigofera tinctoria* leaves had astounding genoprotective movement by acquiring a definite mitotic list in prior and then afterward transformation brought about by transfluthrin, for example, 88%. The huge measure of DNA fracture additionally got from the impact of *Indigofera tinctoria* leaves separate on *Allium cepa* root. The genoprotective action of *Indigofera tinctoria* leaves extricate impact tried against by insecticidal, for example, transfluthrin and *Allium cepa* go about as a biomarker. In this work, the impact of *Indigofera tinctoria* fix the transformation brought about by the transfluthrin and hold the mitotic file as gotten before the change. So, presumed that *Indigofera tinctoria* go about as a brilliant freak fix instrument against insecticidal.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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