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Phytochemical screening, antifungal activity and curative impact on *channa punctatus* fish of *Butea Monosperma* (LAM): Flower, leaves and gum

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ABSTRACT

In the first part of experiment, antifungal activity of water and methanol extracts were studied against pathogenic fungi viz., Cryptococcus neoformans, Aspergillus niger, Candida albicans, and Cryptococcus leuteolus. Among the solvents used, both methanol and water extracts were found to be more effective against Cryptococcus neoformans and Cryptococcus leuteolus. Aspergillus niger and Candida albicans does not inhibit by any type of extract. The second part of experiment performed on fish. In this first set of experiment was treated as control. In the second set of experiment fishes were exposed to 0.15gm/^{-1} concentration of a Parthenium flower extract in aquatic medium for 30 days with a regular interval of 7 days. Due to this histological damage has been observed in gonads which affect the fish production. In the third set of experiment, (0.15gm/l^{-1}) concentrations of water extract of Butea monosperma gum were run simultaneously with the same concentration of a Parthenium flower extract (0.15gm/T^{1}) in aquatic medium for 30 days with a regular interval of 7 days. In mixed treatment Butea monosperma gum extract significantly reduce the histological damage observed due to Parthenium flower extract and help for healthy reproduction. In the fourth set of experiment fish were exposed to (0.15gm/l^{-1}) concentrations of water extract of Butea monosperma gum only and we observe that better histological changes as compared to first set of experiment i.e. Control. Results suggested that under present experimental conditions Butea monosperma extracts acts as an antifungal agents and exhibit strong reproductive activity in above fish model, which could further contribute to study its benefit in humans.

Key words: Antifungal, Reproductive activity, Channa punctatus, Butea monosperma.

INTRODUCTION

Butea monosperma Lam. (Fabaceae) also known as flame of the forest, is a deciduous tree with a somewhat crooked trunk, up to 15m in height and 1.6-2.0m in girth; commonly found throughout India, except in the arid regions. In the literature, *B. monosperma* is ascribed to have many medicinal properties. The flowers are reported to possess astringent, diuretic, depurative,

aphrodisiac and tonic properties. They are also effective in leprosy, leucorrhoea and gout. A decoction of the flowers is given in diarrhoea and to puerperal women. The bark is reported to possess astringent, bitter, pungent, alterative, aphrodisiac and anthelmintic properties. The roots are useful in elephantiasis, and in curing night blindness and other defects of sight. They are also reported to cause temporary sterility in women. The gum of it is a powerful astringent; it is given internally for diarrhea and dysentery, phthisis and haemorrhage from stomach and bladder. A solution of the gum is applied to bruises and erysipelatous inflammations and ringworm [1]. The gum is also applied, when fresh, to ulcers and relaxed sore – throats. The seeds, when pounded with lemon juice and applied to the skin, act as a rubefacient. When made into paste, they are used as a remedy for ring worm [2].

MATERIALS AND METHODS

1.1. *Plant material*

The leaves, flower, and Gum of *Butea monosperma* plant were collected from the Melghat region of Amravati, District of Maharashtra, India in the month of December – February and it was authenticated by the taxonomists Dr. S. P. Rothe from the Department of botany, Shri Shivaji College Akola. A voucher specimen (ML - 101) was deposited in the herbarium of Department of Botany, Shri Shivaji College, Akola.

1.2. *Extraction and isolation*

The leaves, flower, and Gum of *Butea monosperma* plant were shade dried at room temperature ground in a manual mill to get coarse powder. The powder were kept in the air tight polythene bags and stored at dry place. The powder was extracted by using soxhlet extractor with water and methanol as a solvent. The extracts were concentrated for further studies at 50 °C on hot plate. Test extracts were then dried, crushed and stored in air tight bottle for further study. The water extracts of flower were screened for different phytochemical constituents. All the extracts were characterized for different constituents and screened for antifungal tests.

1.3. *Phytochemical screening*[3,4]

The chemical tests were performed for testing different chemical groups present in water extract of flower.

1.3.1. Test for Sugar

a) Molisch's test: - Positive

The Molisch's reagent was prepared by dissolving 10 gm of α -napthol in 100 ml of 95% alcohol. 1 ml of filtrate was mixed with 2 drops of molisch's reagent. To this solution, 1ml of concentrated sulphuric acid was added from the side of the inclined test tube, so that two acids formed a layer beneath the aqueous solution without mixing with it.

Red brown ring appears at the common surface of the liquids, sugar is present.

b) Iodine Test: - Negative

The iodine solution was prepared by dissolving 2 gm of iodine and 3 gm of potassium iodide in 100 ml water.

1ml of above filtrate was mixed with iodine solution, blue colour was not appears, sugar is absent.

1.3.2. Test for Flavonoids

a) Shinoda test: - Positive

The extracts were dissolved in alcohol. One piece of magnesium fallowed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour demonstrated the presence of flavonoids.

b) H₂SO₄ test: - Negative

A few mg of extract was mixed with dilute sulphuric acid. No development of yellow to crimson colour shows the absence of flavonoids

1.3.3. Test for Sterols

a) Salkowaski test: - Positive

10 mg of extract was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red colour in chloroform layer indicated the presence of sterols.

b) Vanillin test: - Positive

Few mg of extract was dissolved in vanillin solution (100 mg of vanillin dissolved in concentrated sulphuric acid-ethanol in proportion of 4:1) development of Blue to brown colour indicates the presence of sterols.

1.3.4. Test for Alkaloids

a) Wagner's Reagent test: - Negative

It was prepared by dissolving 1.27 gm of iodine and 2 g of potassium iodide in 5 ml of water and value up the volume to 100 ml with distilled water.

When few drops of this reagent were added to the filtrate it does not shows reddish brown precipitate indicates the absence of alkaloids.

b) Mayer's reagent test: - Negative

It was prepared by dissolving 1.36 gm of mercuric chloride in 60 ml distilled water, added it to a solution of 5 gm of potassium iodide in 20 ml distilled water making volume to 100ml.

To a 1ml of test filtrate in a watch glass, a few drops of above reagent were added. No formation of cream coloured precipitate shows the absence of alkaloids.

1.3.5. Test for Tannin

a) Ferric chloride test :- Positive

5 ml of filtrate was allowed to react with 1ml of 5% ferric chloride solution. Dark green or deep blue colour is obtained, tannin is present.

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b) Lead acetate test: - Negative

5 ml of filtrate was treated with 1 ml of 10% lead acetate solution in water. No yellow colour precipitation demonstrated the absence of tannins.

1.3.6. Test for Protein and Amino acid

a) Biuret test: - Negative

1 ml of filtrate was taken in water and 1ml of 4% ($CuSO_4$) copper sulphate was added to it violet or pink colour not formed, proteins are absent.

b) Xanthoprotein test: - Negative

A little of amount of filtrate was taken in 2ml of water and 0.5ml of concentrated nitric acid was added do it. Yellow colour not obtained proteins are absent.

1.3.7. Test for Resin: - Positive

a) NaOH test: - Positive

Few mg of extract was treated with caustic soda a red colour was developed resins are present.

1.4. Chemicals

Mueller Hinton agar, SDA and Antifungal Assay Agar (Himedia Lab); Methanol (Ranbaxy laboratories Ltd. S.A.S. Nagar); Standard Discs of Nystatin and Clotrimazole (Himedia Lab); Nutrient Broth, PDA (Himedia Lab)

1.5. Microorganism used

The microorganisms which are used for the antifungal activity were brought from National Chemical Laboratory Pune. The fungi related with skin diseases were selected. These are as follows.

Fungi: Cryptococcus neoformans NCIM No.3542, Aspergillus niger NCIM No.620, Candida albicans NCIM No.3471, ATCC No.10231, Cryptococcus leuteolus NCIM No.3238.

1.6. Antifungal assay

The methanol, and water extracts were examined for their antifungal potency by Cup plate agar method [4] against four fungal species *viz.*, *Cryptococcus neoformans*, *Aspergillus niger*, *Candida albicans*, and *Cryptococcus leuteolus* which generally related to skin diseases. Petri plates were prepared with 25ml sterile SDA. A sterile cork borer (8 mm) was used to make wells in each plate for extracts.1 ml inoculums suspension was swabbed uniformly over the agar medium to get uniform distribution of bacteria. These plates were labelled and 100µl of each plant extracts (at concentration of 200 mg/ml) was added aseptically into the well. The petri plates were then incubated at 27°C for 48 hrs during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The negative control was prepared using respective solvent. Nystatin disc (100 units/disc) and Clotrimazole disc (10 mcg/disc) were used as positive control. The zone of inhibition was recorded in millimetres by using Himedia Zone Reader Scale.

	Concentration	Inhibitory zones in mm			
Extracts		Cryptococcus neoformans	Aspergillus niger	Candida albicans	Cryptococcus leuteolus
1. Water extract of leaves	200 mg/ml	16mm			
2. Water extract of Gum	200 mg/ml				
3. Water extract of Flower	200 mg/ml				20mm
4. Acid content of Gum (H2O Extract)	200 mg/ml	22mm			20mm
5. Acid content of Flower (H2O Extract)	200 mg/ml				28mm
6. Non Acid content of Gum (H2O Extract)	200 mg/ml				
7. Methanol extract of leaves	200 mg/ml	22mm			20mm
8. Methanol extract of Gum	200 mg/ml				24mm
9. Methanol extract of Flower	200 mg/ml				
10. Methanol (Control)					
11. Nystatin disc (Control)		20mm			
12. Clotrimazole disc (Control)					

Table No. 1:- Antifungal activity of different extract of the plant Butea monosperma

2. Materials and Methods for Fish experiment

Live specimen of *Channa punctatus* fishes of both the sexes having similar weight and length were purchased from the local fish market of Akola and brought to the laboratory in the wide mouthed plastic container. After thorough washing in the tap water these were put for deep treatment in 2% KMnO₄ solution. Apparently healthy fishes $(10\pm1\text{cm} \text{ and } 30-40 \text{ gm})$ were acclimatized to the laboratory condition for 7 days in aged tap water (5). They were fed twice a day with pieces of earthworms, eggs and special floating type fish food at regular interval of 12hr. Water in aquarium was changed after every 48 hours. Fecal matter, scales and any wastes of the fishes were removed as and when needed. For this purpose four aquaria were setup, each having 8 healthy and live specimens of fish in aquarium condition. These four aquariums were classified as follows.

Aquarium No. 1 was used as a control in which fishes were maintained as it is in aquarium condition with regular food supply and necessary maintenance. In aquarium No. 2 and 3 Fishes were exposed to the toxicant *Parthenium hysterophorus* (6) flowers extract (0.15gm/l^{-1}) for 30 days of exposure period with regular interval of 7 days. In aquarium No. 3 the induced fishes were simultaneously treated with the water extract of *Butea monosperma* gum (0.15gm/l^{-1}) .

In aquarium No. 4 Fishes were treated with the water extract of *Butea monosperma* gum without inducing them with the toxicant *Parthenium hysterophorus* flowers extract. For all the purposes 30 litre water was taken in each aquarium. At the end of the exposure periods, both control and test fishes of both the sexes were taken out of aquaria and dissected, the testis and ovary were quickly taken out and initially fixed in Bouin's fluid, then dehydrated with alcoholic grade and processed for paraffin embedding. Tissue sections of 6 µm thickness were cut transversally, passed through descending and ascending series of graded alcohol and stained with haematoxylin and eosin for photomicrography. Significant findings were recorded by NIKONHFX-DX trinocular microscope and NIKON-FX-35-DX camera. The changes in exposed sections of Gonads were compared with those of the control sections.

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Sr. No.	Time interval Date	Aqarium1 (Control)	Aqarium2 (Induced)	Aqarium3 (Induced+ treated)	Aqarium4 (Treated)
1.	07.01.2011	Bring the fishes	Bring the fishes	Bring the fishes	Bring the fishes
2.	07.01.2011	As it is	As it is	As it is	As it is
3.	14.01.2011	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight
4.	14.01.2011	As it is	Induced with <i>Parthenium</i> extract (0.15gm/l^{-1})	Induced with <i>Parthenium extract</i> (0.15gm/l^{-1}) and treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l^{-1})	Treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l ⁻¹)
5.	21.01.2011	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight
6.	21.01.2011	As it is	Induced with Parthenium extract (0.15gm/l^{-1})	Induced with <i>Parthenium extract</i> (0.15gm/l^{-1}) and treated with <i>B. monosperma</i> Gum extract (0.15gm/l^{-1})	Treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l ⁻¹)
6.	28.01.2011	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight
7.	28.01.2011	As it is	Induced with <i>Parthenium</i> extract (0.15gm/I^{-1})	Induced with <i>Parthenium extract</i> (0.15gm/l ⁻¹) and treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l ⁻¹)	Treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l ⁻¹)
8.	04.02.2011	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight	sacrifice two fishes out of eight
9.	04.02.2011	As it is	Induced with <i>Parthenium</i> extract (0.15gm/l^{-1})	Induced with <i>Parthenium extract</i> (0.15gm/l ⁻¹) and treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l ⁻¹)	Treated with <i>B</i> . <i>monosperma</i> Gum extract (0.15gm/l ⁻¹)

Table 2:- Table shows the experimental setup

Antifungal Tests

The results obtained for the antifungal tests performed on different solvent extracts of *B. monosperma* are presented (Table 1). Among the solvents used, both methanol and water extracts were found to be more effective against *Cryptococcus neoformans* and *Cryptococcus leuteolus. Aspergillus niger* and *Candida albicans* does not inhibit by any type of extract. Activities of the various extracts were comparable to those of standard antifungal agent Nystatin and Clotrimazole as control. In the present study four different fungal strains were used to screen possible antifungal activity related to skin diseases of *B. monosperma* extracts. A result clearly indicates that extracts showed significant antifungal activity. So it is expected that they could be used to treat infections and diseases caused by these organisms.

RESULTS AND DISCUSSION

Fish experiment

2.1. Histopathological observations of gonads

Testes:-Testes are paired, elongated and situated on ventral side of the kidney in the posterior region of the abdominal cavity. Histologically the testes are composed of a large number of seminiferous tubules, which are closely bound together by a thin layer of connective tissue. The tubules open into a spermatic duct, which is generally lined by secretery epithelium. The spaces between the lobules are filled with connective tissue, blood capillaries and interstitial cells

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(Leydig cells). During the growth period, the resting germ cells become active and various stages of spermatogenesis is seen such as; primary spermatocytes, secondary spermatocytes, spermatids and sperms (Fig. 1).

Fish exposed to the toxicant *Parthenium hysterophorus* flowers extract for different exposure periods showed considerable degree of alteration in the histoanatomy of the gonads. These changes were profound and the degree of changes in histoanatomy showed variation during different exposure periods. The seminiferous tubules are generally of varying shapes and sizes. Each tubule has a definite, thin fibrous wall. The testes of *C. punctatus* have shown significant changes on exposure to test chemical *Parthenium hysterophorus* flowers extract. After 7, 15 days of initial exposure period there is no significant changes were observed. But after 30 days of exposure period, appearance of a large number of intertubular vacuoles was observed (Fig. 2).

In aquarium no. 3, fishes were exposed to sub-lethal concentration of the toxicant *Parthenium hysterophorus* flowers extract along with water extract of *Butea monosperma* gum for different exposure periods. After 30 days of exposure period, the testes showed the recovery of vacuolization of tubular cells and no distortion of seminiferous cells, tubular cells (Fig. 3). It showed the resemblance to that of control fish section.

In aquarium no. 4, fishes were exposed to water extract of *Butea monosperma* gum for different exposure periods with regular interval of 7 days without inducing them to the toxicant *Parthenium hysterophorus* flowers extract. After 30 days of exposure period the section of testes showed somewhat betterment to those of control fish section.



Scheme No. 2: Figures showing the section of testes after experimental treatment

Fig. 1. Section of testis of control fish showing seminiferous tubules

Fig. 2. Section of testis of fish showing intertubular vacuoles after treated with toxicant Parthenium extract

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19. 5 Section of tesus of fish treated with Parthenium extract along with *Butea monosperma* gum extracts showing recovery of intertubular vacuoles.

Fig. 4 Section of testis of fish treated with Butea monosperma gum extracts showing somewhat betterment than those of control fish section

Ovary: - Normal ovaries of *C. punctatus* are elongated sac like structure lying ventrally to the kidney in the abdominal cavity. The wall of the ovary is fairly thick during the non-breeding season. It consists of three layers 1) An outermost, thin peritoneum 2) Thick, tunica albuginea 3) The germinal epithelium that project in to ovocoel in the form of lamellae, which are seats for the development of oocyte.

In the months of December, January & February phase ovaries are small, thin thread like translucent, pale white in color, having nests of oogonia. The section showed various stages of development each oogonia passes through series of developmental stages such as oogonic stage, early perinucleolus stage, late perinucleolus stage, yolk vesicle stage, and migrating nucleus (Fig. 1)

The ovaries of *C. punctatus* had shown significant changes on exposure to test chemical *Parthenium hysterophorus* flowers extract. After 7, 15 days of initial exposure period there was no significant changes were observed. After 30 days of exposure period the ovaries showed, oocyte with de-shaped, disrupted follicular epithelial cells. Nucleolus showed condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells caused vacuolation (Fig. 2).

In aquarium no. 3, fishes were exposed to sub-lethal concentration of the toxicant *Parthenium hysterophorus* flowers extract along with water extract of *Butea monosperma* gum for different exposure periods. After 30 days of exposure period the ovaries showed the recovery of developmental stages such as oogonic stage, early perinuclear stage (nucleoli arranged peripherally), cytoplasm and nucleus showed in normal position as shown in fig.3.



Scheme No. 3: Figures showing the section of ovaries after experimental treatment

Fig. 1. Section of Ovary of control fish

Fig. 2. Section of ovary of fish treated with Parthenium extract showing vacuolization and nucleolus condensation of crescent shaped dark granules at one side.



Fig. 3. Section of ovary of fish treated with Parthenium extract along with Butea monosperma gum extract showing recovery in nests of oogonia.

Fig. 4 Section of ovary of fish treated with Butea monosperma gum extracts showing somewhat betterment than those of control fish section.

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In aquarium no. 4, fishes were exposed to water extract of *Butea monosperma* gum for different exposure periods with regular interval of 7 days without inducing them to the toxicant *Parthenium hysterophorus* flowers extract. After 30 days of exposure period the section of ovaries showed somewhat betterment to those of control fish section.

The overall result suggested that under present experimental conditions water extract of *Butea monosperma* gum exhibit strong power to prevent and protect the histological damage observed in gonads and hence it may be used for healthy reproductive activity in this fish model, which could further contribute to study its benefit in humans.

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