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PHYTOCHEMICAL ANALYSIS AND HEPATOPROTECTIVE SCREENING OF *BUTEA MONOSPERMA* (LAM)

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ABSTRACT

Butea monosperma (Lam), is very common moderate sized widely distributed tree belongs to Kuntze (Syn. *Butea frondosa*; Family Fabaceae), which is deciduous, Burma and Ceylon, popularly known as 'dhak' or 'palas', commonly known as *'Flame of forest'* throughout the India. Fresh leaves and flowers of the plant were collected, dried, homogenized and extracted using 70% Ethanol and distilled water. Phytochemical analysis of alcoholic extract gave positive results for steroids, triterpinoids, reducing sugars, alkaloids, phenolic compounds, flavonoids and aqueous extract gave positive results for tannins. Triterpinoids, reducing sugars, alkaloids, phenolic compounds and flavonoids. Hepatoprotective activity was assay on cultured hepatocytes of mice to conclude that aqueous extract of both leaves and flower has ability to protect hepatocytes from CCl_4 induced stress.

KEYWORDS

Butea monosperma, Phytochemical, Flavonoids, Sterols and Hepatoprotective.

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INTRODUCTION

Plants are the a rich source of organic compounds, many of which have been used for medicinal purposes and many phytochemicals could serve as lead for the development of novel agents having high potency against many pathological disorders and many drug resistance microorganisms in the coming years[1].

Butea monosperma (Lam) is commonly known as Flame of forest due to its gorgeous canopy of scarlet flowers which looks like a flame[2]. Belong to the family Fabaceae[3]. It is locally called as palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino, and Nourouc and is common throughout India, Burma and Ceylon except in very acrid parts. Generally it grows

gregariously on open grasslands and scattered in mixed forest.

It is an erect tree 12-15 m high with crooked trunk and irregular branch's, bark rough, ash coloured, young parts downy[4]. Leaves are 3-foliate, petioles 10-15cm long, stipules linear lanceo late. Leaflets curvaceous (the terminal 10-20cm long, broadly ovate from acuneate base, the lateral smaller, 10-15 by 7.5-10cm, obliquely rounded at the base, equilateral, the lower side the larger), all obtuse, labours above when old, finely silky and conspicuously reticulately veined beneath; petioles 6mm long, stout-stipules sub late, deciduous. Flowers are large, in a rigid racemes 15cm long, 3 flowers together form the tumid nodes of the dark olive-green velvety rachis: pedicels about twice as long as the calyx, densely brown-velvety: bracts and bracteoles small, deciduous. Calyx 13 mm long, dark olive-green, densely velvety outside, clothed with silky hairs within teeth short, the 2 upper connate, the 3 lower equal, deltoid. Corolla 3.8-5cm long, clothed outside with silky, silvery hairs, orange or salmon coloured: standard 2.5 cm broad: keel semicircular, beaked, veined. Pods stalked 12.5-20 by 2.5-5cm, thickened at the sutures, reticulately veined - canes cent: stalked 2 cm long[5].

It was reported by many researchers that plants having woody nature can synthesize and accumulate great variety of phytochemicals specially alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins and lignin[6].

MATERIAL AND METHODS

Collection of plant materials

The Flowers and Leaves were collected from the *Butea monosperma* tree from Barkatullah University campus. It was ensured that the plant were healthy and uninfected. The plant material were taxonomically identified at Biotechnology department Barkatullah University, Bhopal. (Madhya Pradesh) India. The leaves and flowers were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves and flowers thoroughly and dried in shade.

Preparation of leaf and flower extracts

Ethanol Extract

Butea monosperma leaves and flowers (50g) were ground into fine powder using a stainless-steel grinder, and deep in 70% ethanol (200ml) for 72 hours[7] then extracted through shoxelet apparatus. The extract was concentrated by a rotary film evaporator.

Aqueous Extract

Butea monosperma leaves and flowers (50g) were ground into fine powder using a stainless-steel grinder, and deep in distilled water (300 ml) and 5ml of chloroform (to avoid bacterial or fungal growth) for 72 hours then extracted through shoxelet apparatus. The filtered extract was concentrated by a rotary film evaporator.

Phytochemical Analysis

Aqueous extract and Ethanol extract was then subjected to phytochemical screening by the following procedures.

Tests for Flavonoids

Shinoda test

Few fragments of magnesium ribbon and conc. HCL was added to the extract, presence was confirmed when pink colour changes to magenta red colour[6].

Zn/HCL reducing test

Zinc dust was added to the extract followed by few drops of HCL shows magenta red colour.

Alkaline reagent test

Extract when treated with sodium hydroxide solution shows increase in the intensity of yellow colour, which becomes colourless on addition of few drops of dilute acid.

Test for Triterpenoids

Salkowski test

Few drops of concentrated sulphuric acid was added to the extract, shaken and on standing lower layer turns golden yellow[6].

Liebermann Burchard Test

Few drops of acetic anhydride was added to the extract, and mixed well. Then 1ml of concentrated sulphuric acid was added from the sides of the test tube, a red colour is produced in the lower layer indicate Triterpenes[6].

Tschugagiu test

0.5g of acetyl chloride and pinch of zinc chloride added to the solution of extract, kept aside for reaction to subside and warmed on water bath, eosin red colour produced.

Briekorn and Brinar test

To the solution of extract, few drops of chlorosuphonic acid in glacial acetic acid (7.3) red colour is produced.

Tests for Glycosides

Baljet's test

The extract was treated with sodium picrate gives yellow to orange colour[6].

Bromine water test

Solution of extract when dissolved in brominoine water gives yellow precipitate.

Raymond's test

Extract solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

Legal's test

Extract solution when treated with Pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

Tests for Anthraquinones

Borntrager's test

Extract was boiled with 5 ml of 10% sulphuric acid for 5mins. Filtered while hot, cooled the filterate shaked gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution of ammonia (10%). Then allow to separate ammoniacal layer aquires rose pink colour due to the presence of anthraquinones.

Tests for Saponines

Foam test

Extract solution when shaken shows the formation of foam, which is stable an least for 15mins.

Tests for Carbohydrates

Molisch's test

Test solution with few drops of Molisch's reagent and 2ml of concentration H_2SO_4 added showly from the sides of the test tube shows a purple ring at the junction of 2 liquids.

Barfoed's test

Extract solution treated with Barfoed's reagent, on boiling on a water bath shows brick red colour precipitate.

Benedict's test

The extract solution treated with Benedict's reagent and boiling on water bath shows reddish brown precipitate.

Fehling's test

The extract solution when heated with equal volume Fehling's A and B solutions, gives orange red precipitate presence of reducing sugars.

Selivinoff's test

A crystal of resorcinol is added to the extract solution and warmed on a water bath equal volume of conc. HCL a rose colour is produced that showed ketone is present.

Tests for pentoses

Heat the extract solution with an equal volume of HCL containing little phloroglucinol. Formation of red colour lidicates the present pentoses.

Tests for Alkaloids[6]

Mayer's test

Extract solution with Mayer's reagent (Potassium Mercuric iodide) gives cream coloured precipitate.

Hager's test

The acidic solution with Hager's reagent (Saturated picric acid solution) gives yellow precipitate.

Dragendorff"'s test

The acidic solution with Drogendroff's reagent (Potassium bismuth iodide) shows reddish brown precipitate.

Tests for Phytosterols

Salkowaski Test

To the extract solution added few drops of conc. H2SO4 shaken and allowed to stand, lower layer turn red indicating the presence of sterols.

Liebermann - Burchard test

The extract solution treated with few drops of acetic anhydride and mixed, when conc. H_2SO4 is added from the sides of the test tube, it show a brown ring at the junction of the two layers and the upper layer turns green.

Sulphur test

Sulphur when added in to the extract solution, it sinks in it.

Tests for Phenolics

FeCl3 test

Extract solution treated with few drops of $FeCl_3$ solution gives dark colour.

Gelatin test

Extract solution treated with gelatin gives white precipitate.

Tests for Proteins

Millon's test

Extract solution when treated with Millon's reagent and heated on a water bath, Protein is stained red on warming.

Xanthoproteic test

Extract solution treated with conc. HNO_3 and boiled gives yellow precipitate.

Biuret test

Test solution treated with 40% NaOH and dilute $CuSO_4$ solution gives blue colour.

Tests for Amino acids and Imides

Ninhydrin test

Extract solution treated with Ninhydirn reagent gives blue colour.

Tests for Phytosterols

Salkowaski Test

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Liebermann - Burchard test

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Sulphur test

Sulphur when added in to the extract solution, it sinks in it

Volatile oils

2ml of both extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is not formed it means volatile oils are absent.

Cell preparation

In present study single cell suspension of hepatocytes was prepared from liver of adult Wister mice through mechanical shearing (Ursal *et al*, 2014), and cultured in culture petriplates in RPMI1640 with 2% agarose to provide 2D frame, supplememted with 5% FBS and streptomycin (100µg) in CO_2 incubator with 5% CO_2 at 35^oC or 24 hrs, then cells were subjected to CCl_4 treatment for toxicity (Deshpande and Gothalwal, 2015)[8].

Determination of Mitochondrial Synthesis by Micro culture Tetrazolium (MTA Assay)

The ability of the cells to survive a toxic treatment has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTA to a blue formazan derivative by living cells in clearly a very effective principle on which the assay is based (Freshney, 2000)[9].

***In vitro* hepatoprotective activity against CCl_4 induced toxicity**

Below the CTC_{50} value two dose levels were selected for each extract and used for further studies. In present study modified method of Vijayan *et al*, 2002 monolayer was washed once and treated with 100µl of different drug concentrations for 24 hrs. After 24 hrs of pretreatment with the extracts, the cells were challenged with CCl_4 (15mM) where 100µl of different drug concentration and 100µl of CCl_4 was added. The plates were then incubated at 37^oC for further 24 hours in 5% CO_2 atmosphere (Deshpande and Gothalwal, 2015)[8]. Microscopic examination was carried out and observations were recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTA in RPMI 1640 was added to each well. The supernatant was removed and 50µl of propanol was added to solubilize the formed formazan.

Morphological analysis

Cells were examined microscopically for any morphological change induced by treatment of drug and CCl_4.

RESULTS AND DISCUSSION

Results of phytochemical screening of *Butea monosperma* are given in Table No.1. Liver injuries induced by CCL_4 are the best characterized system of xenobiotic- induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and or hepatoprotective activities of drugs (Clawson 1989[10], Lin *et al*, 2002[11]). Percentage of viable cells exposed to various extracts, CCl_4, and Liv52 were given in Table No.2. It was suggested that, CCl_4 get accumulated and

metabolically activated in hepatic parenchyma cells which is cytochrome P450 dependent monooxygenases to form trichloromethyl radical (CCl_3). The CCl_3 radical attack on cellular proteins to alkylates and other macromolecules simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, resulted in liver damage. Thus it was suggested by Bishayee *et al*, 1995[12], that antioxidant or free radical generation inhibition is important to protect liver against CCl_4 induced damage. These findings were supported by Muthu *et al*, (2008)[13] through various enzyme assays. CCl_4 induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST) which results in liver damage.

Results indicates that alcoholic extract of leaves gives protection to hepatocytes with 76.32±1.72 percentage while aqueous extract of leaves and Live52 shows 75.32±1.42 and 78.83±1.94 respectively. Aqueous and ethanolic extract flower of *Butea monosperma* shows 55.63±1.08 and 58.53±1.23 respectively.

Table No.1: Results of phytoconstituents

S.No	Parameter	Flower	Leaf	Flower	Leaf
		Aqueous Extract		Ethanolic Extract	
1	Saponins	-	-	+	+
2	Tannins	+	+	+	+
3	Reducing sugars	+	+	+	+
4	Glycosides	-	+	-	+
5	Alkaloids	+	+	+	+
6	Flavonoids				
7	Volatile oils	-	-	-	-
8	Sterols	-	-	+	+

Table No.2: Results of effect of various drugs on liver cells *in vitro*

S.No	Cells and treatment given	Amount of drug ($\mu g\ ml^{-1}$)	No. of viable cells in %
1	Control	-	100
2	Live52	230	78.83±1.94
3	CCL_4	15mM	32.21±1.02
4	Aqueous extract of flower	750	55.63±1.08
5	Aqueous extract of leave	550	75.56±1.42
6	Alcoholic extract of flower	850	58.53±1.23
7	Alcoholic extract of leave	600	76.32±1.72

Figure No.1: *Butea monosperma* tree in Barkatullah University

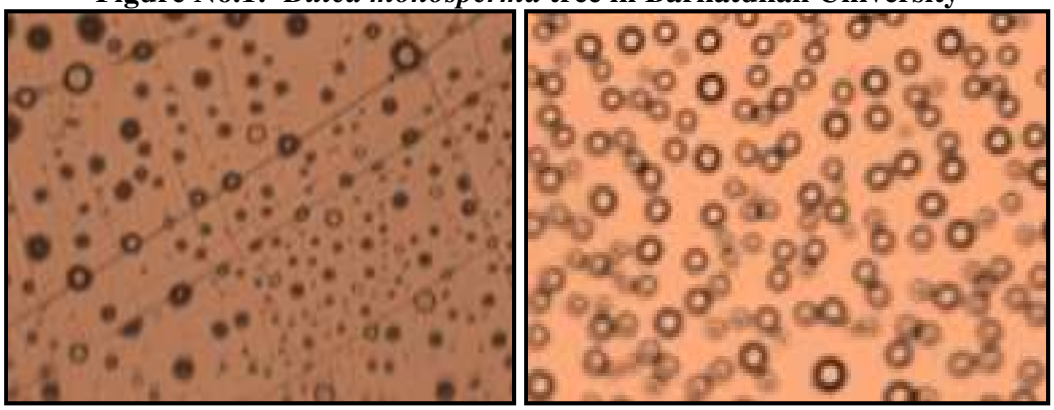

Figure No.2: (a): Dead hepatocytes at 40x magnification (b): Viable hepatocytes at 40x magnification, Cells were stained with tryphan blue stain

CONCLUSION

Phytochemical analysis confirms the presence of many phytoconstituents of extracts of flowers and leaves. *In Vitro* study on hepatocytes confirms the effectiveness of ethanolic extract and aqueous extract of leaves *Butea monosperma* compare to Liv52 which should be further scientifically validated in terms of amount of active phytochemical, efficacy of molecule. Extensive study is needed so, that novel drug apart from Liv52 can be explored and formulated.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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