



IN-VITRO PHYTOCHEMICAL SCREENING OF THE FLOWER EXTRACTS OF *BUTEA MONOSPEMA* (LAM.) TAUB

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ABSTRACT

Aqueous and acetone extracts of the plant *Butea monosperma* flowers of the family Leguminosae - Papilionaceae were prepared by adding 25 g of fresh flowers to 100 ml of these solvents. The constituents were extracted by soxhlet extractor for 24 h. After incubation, the extracts were collected and stored at 4°C. In the present study, all the seven groups of the important phytochemicals, viz., cardiac glycosides, steroids, alkaloids, flavonoids, terpenoids, tannins and saponins, were screened from the plant *Butea monosperma* flowers. Their presence was confirmed through in vitro phytochemical screening of aqueous and acetone extracts of flower. The analysis for the same was carried out by qualitative tests for which the standard protocol was followed. The presence or absence of the phyto-constituents depended upon the solvent used and physiological property of the flowers. From these results, it can be concluded that the *B. monosperma* flower extracts may be used as broad-spectrum bioactive agents after extensive investigation.

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INTRODUCTION

A large number of Indian medicinal plants have been the important components of traditional medicinal system, and also in modern pharmaceuticals. Their biologically active functions and medicinal value is due to the presence of a wide range of chemical compounds in different parts of the plants. Most of such compounds act as natural antioxidants, and help in the disease treatments and early ageing (Hussain and Kumar 2016). Medicinal plants contain numerous active ingredients that may be potentially useful for the development of therapeutic agents. The identification and isolation of phytochemical groups and/or single chemical entities from them are, hence, crucial for drug discovery as these entities often work as individual agents or as a collective group of phytochemicals (purified extracts) to achieve the desired therapeutic effect.

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However, to assess their quality, standardisation of these plant parts needs to be carried out, which includes a series of tests to determine the quality, quantity and the purity of the phytochemicals or the extracts, along with the measure of contaminants or foreign matter present in them (Pradhan et al. 2015; Vaidya and Pandita 2017). The bioactive compounds of plants have a wide range of biological functions including, antimicrobial, antioxidant, anti-inflammatory activities (Burt 2004; Chanda et al. 2010). Plants have to adapt to the changing environmental conditions for their survival of existence. The oxidative environment presents a range of free radicals including superoxide, hydroxyl radical, nitric oxide and peroxynitrite, for living organisms to deal with. Many evidences are existing to explain the role of free radicals in the development of various diseases including cancer, neurodegeneration and some inflammatory diseases (Halliwell 2006, 2007; Ferguson 2010, Munawar and Rao 2017). *B. monosperma* (Palas) Leaves are used to cure boils, pimples, haemorrhage, it is astringent, diuretic, anti-diabetic and arrest bleeding (Akhtar et al. 2010; Ahmed et al. 2012). Flowers are aphrodisiac and tonic properties. Flowers have anti-inflammatory (Muralidhar et al. 2010a; Muralidhar et al.

2010b) and anti-cancer activity (Lau *et al.* 2010; Rasheed *et al.* 2010). Seed are used for anti-implantation and anti-ovulatory properties. Bark is having anti-microbial properties (Tambekar and Khante 2010), anti-fungal activity (Singh 2011) and used in tumors (Patil *et al.* 2006), bleeding piles, ulcers. Its medicinal properties are enshrined in ancient Indian scriptures with almost each part of the plant namely roots, stem, bark, leaves, flowers, fruits, seeds and gum are used in Ayurvedic and Unani medicine (Mazumder *et al.* 2011, Mishra 2016).

Plant *Butea monosperma* (Lam.) Taub. Botanical

Description: *Butea monosperma* (Palas) is a medium-sized deciduous tree belongs to family Leguminosae- Papilionaeae. It is a small to medium-sized, slow-growing, deciduous trees, to 10 m high, bole crooked, irregular; bark 5-6 mm thick, grey to greyish-brown; exudation red; branchlets densely tomentose. Leaves trifoliate, alternate; stipules small, lateral, cauducous; rachis 12-20 cm long, stout, pubescent, pulvinate; stipelssubulate; petiolule 5-10 mm long, stout, pubescent; lateral leaflets 8.8-13.7 x 5.5-11 cm, broadly oblong-ovate or suborbicular, base oblique, apex obtuse, terminal leaflet 11-15 x 12.5-15 cm, widely rhomboid, base obtuse, apex emarginate, silky pubescent on both sides when young, glabrous above, silky pubescent beneath when mature, margin entire, coriaceous; lateral nerves 4-8 pairs, pinnate, prominent; intercostae scalariform, prominent. Flowers bisexual, 5 cm long, bright red, in terminal or axillary, densely fascicled, racemes; calyx broadly campanulate, teeth 5, deltoid, short, upper 2 connate, velvety; corolla much exserted; petals 5, standard petal 5 x 2.5 cm, lanceolate, clawed, wings falcate 4.5 x 1.5 cm adnate to keel, keel united 4.5 x 3 cm, curved; stamens 9 + 1; vexillary stamens free; anthers uniform; ovary 2.5 cm, inferior, 1-celled, ovules 2; style long, incurved, beardless; stigma small. Fruit a pod, 12.5-28 cm long, oblong, the base flat, wing-like and indehiscent, the tip splitting round the apical seed; seed obovate, compressed. (India Biodiversity Portal 2021).

Chemical constituents of *Butea monosperma*: Leaves contains alkaloids (Euphane trieterpenoid and pterocran), Flowers having (butrin, butein, butin, isobutin, coreopsin, monospermoside and their isoderivatives and sulphurein, palastrin). Seed contains palasonin, d-mecantharidinproteolytic and lipolytic enzymes, -amyirin, -sitosterol and alkaloid monospermine glycerides of stearic, palmitic, linoceric, oleic and linoleic Acids. Bark contains tannins and gum (*Butea* gum), leucocyanidin, it tetramer, procyanidin, allic acid and mucilaginous material (Dubey *et al.* 2012; Mishra 2016).

Ayurvedic context of *Butea monosperma*: *Butea monosperma* (Palash) has a great impact as a medicinal herb used in Ayurvedic medicine. Its characteristic is well defined in Charaka Samhita, Susruta Samhita, Astanga Sangraha, Astanga Hridaya Vedas, and Upanisads. In Charaka Samhita, the plant is defined in Mahakasaya. In Susruta Samhita, Palash is considered as Ambasthadi, Nyagrodhadi, Muskakadi and Rodhradi. Similarly, in Astanga Hridaya and Susruta Samhita Palash is also described in Ambasthadi, Muskakadi, Nyagrodhadi Gana and Rodhradi. In Astanga Sangraha, Vagbhata mentioned Palash in Asanadi, Rodhradi, Muskakadi, Ambasthadi and Nyagrodhadi Gana. Palash also has detailed description in Samhita's such as Harita Samhita, Bhela Samhita, Sharangadhar Samhita and Kasyapa Samhita. It is also stated in Cikitsa granthas as Gadani-graha, Bhaishajya Ratnavali, Bhavaprakasha Samhita and Cakradutta. Nighantus

have defined the goodness of Palash in various disease for example the rasa of Palash is useful in kasaya and tikta. According to Bhavaprakash Nighantu the fruit part of the *B. monosperma* is applicable in Krimi, Arsa and Vatakaphaja rogas (Tiwari *et al.* 2019)

Flowers of *Butea monosperma*: Flowers are astringent, sweet, cooling, constipating, aphrodisiac, haemostatic, diuretic, febrifuge, depurative and tonic. They are useful in diarrhea, haemorrhoids, menorrhagia, fever, leprosy, skin diseases, swelling, hyperdipsia, haemoptysis, arthritis, burning sensation and bone fracture. The Chemical constituents of flower is seven flavonoid glycosides like butrin, isobutrin, monospermoside, isomonospermoside, coreopsin, isocoreopsin and sulphurein. With increasing demand for safer drugs attention has been drawn to the quality, safety, efficacy and standards of the Ayurvedic drugs (Gavit *et al.* 2015). Hence, there is a need for standardization and development of reliable quality protocols for Ayurvedic drugs using modern techniques of analysis. Keeping this in view, present study was carried out to evaluate *In-vitro* phytochemical screening of the flower extracts of *Butea monosperma* (Lam.) Taub.

MATERIALS AND METHODS

Collection and authentication of plant material: The flowers of *Butea monosperma* were collected in the month of March 2020 from six different locations in Surgana Tehsil of Nashik District, Maharashtra India. These plants were taxonomically identified, authenticated and the voucher specimen has been deposited in the herbarium section, Department of Botany, MGU's ASC College Surgana, District Nashik, (MH).

Processing of Plant Materials: The plant Materials were cleaned and fresh plant material (whole flower) were taken. The samples were kept in a sterile vessel with suitable marking until needed for further analysis.

Preparation of flower Extract: Solvent extraction of crude whole flower extract was made ready by Soxhlet extraction techniques. About 20 gm of fresh plant material was equally packed into a thimble and extracted with 250 ml of two solvents one by one. Solvents used were acetone and distilled water as per polarity. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor emerge as colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for their further use in phytochemical evaluation (Hait *et al.* 2019).

***In-Vitro* phytochemical screening:** The extract was tested for the presence of bioactive compounds by using following standard methods (Harborne 1973; Trease and Evans 1989; Sofowra 1993; Kokate 1994; Mishra *et al.* 2011).

Test for Amino Acids: 2 ml of solvent extract was mixed with 2ml ninhydrin reagent and kept in hot water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Carbohydrates: 2 ml of methanolic extract was mixed with 2 drops of Molisch's reagent and shake well. Add 2 ml of concentrated sulphuric acid in the sides of the test tube.

A reddish violet color ring appeared at the junction of the two layers immediately indicated the presence of carbohydrates in the sample.

Test for Alkaloids: 1 ml extract was mixed with 1% HCl and 6 drops of Mayer's reagent and Dragendorff's reagent. A turbidity or precipitation indicated the presence of alkaloids in the sample.

Test for Steroids: 2 ml of acetic anhydride was mixed with 0.5 ml solvent extract and further added with 2 ml concentrated sulfuric acid. The color change from violet to blue or green indicates the presence of steroids.

Test for Cardiac Glycosides: 5 ml of solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution already. This solution is further under layered with 1ml conc. H₂SO₄. A brown ring on the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

Test for Flavonoids: Aqueous extract was added with 5 ml ammonia solution and conc. H₂SO₄. A yellow coloration confirms the presence of flavonoids which disappears on standing long.

Test for Saponins: Take small amount of extract with 20 ml of distilled water. Agitate the mixture for 15 minutes in graduated cylinder. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins: Take 5 ml of extract with few drops of lead acetate. A yellow precipitate confirms the presence of tannins.

Test for Terpenoids: Take 2 ml solvent extract with 2 ml of chloroform and 3 ml of conc. H₂SO₄ to form a monolayer of reddish brown coloration of the interface revealed presence of terpenoids.

Test for Phenols: 2 ml of extract was mixed with 3 ml of ethanol and a pinch of FeCl₃ to form greenish yellow color showing presence of phenols.

Test for Coumarins: 3 ml of 10% NaOH was mixed with 2 ml of aqueous extract. Formation of yellow color indicates coumarin.

Test for Emodins: 2 ml of NH₄OH and 3ml of benzene was mixed with extract. The appearance of red color indicates presence of emodins.

RESULTS AND DISCUSSION

Plant-derived substances have recently become of great interest owing to their diverse applications. Medicinal plants are the richest bioresources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Doss 2009). The medicinal plants show healing properties are generally due to the presence of various secondary metabolites in their various parts and organs. The aqueous and acetone extracts of flowers of *Butea monosperma* have revealed the presence of amino

Table 1. Preliminary phytochemical tests for plant extracts

Sr. No.	Phytochemicals	Test	Observations
1.	Amino acids	2 ml of extract + 2ml ninhydrin reagent (in hot water bath for 20 min.)	Purple coloration
2.	Carbohydrates	2 ml of extract + 2 drops of Molisch's reagent + 2 ml conc. H ₂ SO ₄	Reddish violet color ring appeared at the junction of the two layers
3.	Alkaloids	1 ml extract + 1% HCl + 6 drops of Mayers and Dragendorff reagent	Turbidity or precipitation
4.	Steroids	0.5 ml solvent extract + 2 ml of acetic anhydride + 2 ml conc. H ₂ SO ₄	Color change from violet to blue or green
5.	Cardiac Glycosides	5 ml of extract + 2 ml glacial acetic acid + 1 drop FeCl ₃ + 1ml conc. H ₂ SO ₄	Brown ring / violet ring / greenish ring
6.	Flavonoids	2ml extract + 5 ml ammonia solution + conc. H ₂ SO ₄ .	Yellow coloration
7.	Saponins	1 ml extract + 20 ml of distilled water (Agitate for 15 min.)	1cm layer of foam
8.	Tannins	5 ml extract + few drops lead acetate	Yellow precipitate
9.	Terpenoids	2 ml extract + 2 ml CHCl ₃ + 3 ml of conc. H ₂ SO ₄	Monolayer of reddish brown coloration of the interface
10.	Phenols	2 ml of extract + 3 ml of ethanol + pinch of FeCl ₃	Greenish yellow color
11.	Coumarins	3 ml 10% NaOH + 2 ml of extract	Yellow coloration
12.	Emodins	2 ml NH ₄ OH + 3ml benzene	Red coloration

Table 2. Results of In-vitro phytochemical screening of *Butea monosperma* flower extracts

Sr. No.	Variable	Aqueous Extract	Acetone Extract
1.	Amino acids	+	-
2.	Carbohydrates	+	-
3.	Alkaloids	+	+
4.	Steroids	-	+
5.	Cardiac Glycosides	-	+
6.	Flavonoids	+	+
7.	Saponins	+	-
8.	Tannins	+	+
9.	Terpenoids	-	+
10.	Phenols	-	+
11.	Coumarins	+	+
12.	Emodins	+	+

(+) = Presence, (-) = Absence

acids, alkaloids, flavonoids, cardiac glycosides, phenols, saponins, sterols, terpenoids, coumarins, emodins and tannins (Table 1). Thus the *in-vitro* phytochemical screening may be useful in the detection of the biologically active principles and may lead to the drug discovery and development. The *in-vitro* phytochemical screening of the flowers of *B. monosperma* is tabulated in Table 1 and Table 2. From the *in-vitro* phytochemical screening of flower of *B. monosperma*, we observed that the aqueous and acetone extracts gave a positive result with Mayer's and Dragendorff's reagent which indicated the presence of alkaloids in both the extracts. Both aqueous and acetone extracts of flowers showed the distinct presence of alkaloids, flavonoids, tannins, coumarins and emodins. Acetone flower extract was found as the good sources of bioactive secondary metabolites. The aqueous extracts of *B. monosperma* flower ascertained the presence of amino acids, carbohydrates, alkaloids, flavonoids, saponins, tannins, coumarins and emodins. Whereas, acetone extract was found to consist of good stores of phytochemicals such as alkaloids, steroids, cardiac glycosides, flavonoids, tannins, phenols,

coumarins, emodins and terpanoids (Table 2). Steroids, cardiac glycosides, terpenoids and phenols were found to be absent in aqueous extract of *B. monosperma* flower whereas, shown presence in acetone extract of flower. Likewise, amino acids, carbohydrates and saponins were found to be absent in acetone extract of *B. monosperma* flower, which were present in aqueous extract of flower. *In-vitro* phytochemical screening of flowers of plant *Butea monosperma* gave positive results for bioactive secondary metabolites. The future need is to characterize and purify the essential therapeutic biomolecules of flowers for drug development including health care products. The scientific communities need to discover the ability of alternate source and to establish pharmaceutical industries for making use of this eminent aid which get wasted each year within the flowering season. The purification of those phytochemicals and backbone of their respective antimicrobial potencies ought to be the prospect direction for examination.

CONCLUSION

Several research confirmed that the presence of secondary metabolites contributes medicinal properties to plants. Therefore, extracts from plant could be seen as an excellent source for beneficial drugs. *In-vitro* preliminary phytochemical screening is useful in the detection of bioactive compounds and subsequently may lead to drug discovery and development. Our findings offer proof that the crude aqueous and acetone extracts of *Butea monosperma* plant has a potential source of natural bioactive metabolites, and this justified its use in folkloric drugs and ethno medicinal purposes.

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