WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

An investigation into the phyto-chemical evaluation and anti-cancerous and anti-inflammatory analysis of leaf extract of Butea monosperma

Roopa P[1], Romana Mirdhe[2]

[1] Department of Botany, S.E.A College of Science, Commerce and Arts, Ektanagar, KR Puram, Bengaluru, India

[2] Department of Botany, Faculty of Sciences, Khaja Bandanawaz University, Kalaburagi, India

*Corresponding author: Roopa P (Email: drroopap.31@gmail.com)

ABSTRACT :

Each day, human civilization confronted with new health challenges, requiring the discovery of innovative solutions. Hence, this study was conducted to reaffirm the therapeutic properties in the leaf extracts of Butea monosperma and it was concluded from the results that the preliminary phytochemical analysis showed presence of alkaloids, carbohydrates, cardiac glucosidase, flavonoids, phenols, phlobataninns, amino acids and proteins and tannins whereas, saponins, steroids, terpenoids, quinones, oxalates, fats and fixed oils were found to be absent on quatitavie analysis using HPLC method it was revealed that phenolics present in the leaf extract of Butea monosperma are /0.1202 g /g of extract and the flavonoids present in the leaf extract of Butea monosperma are 0.201 g/g of extract besides, the leaf extract tested positive for anti-cancerous and anti-inflammatory activity.

Keywords: therapeutic, *Butea monosperma*, phenolics, flavonoids

1.Introduction

The rise in resistant microbes is a major threat to our health infrastructure hence; there is a pressing need to increase our research in the field of Pharmocognosy to find new bio-chemicals which can be used against various diseases in humans and other animals. Butea monosperma is the deciduous tropical plant belonging to the family Fabaceae exquisitely known as Flame of the forest and Bastard Teak. The tree finds lot of religious significance as it is said to have sprung from the feather of falcon impregnated with the soma the beverage of god (Geetha, 2017). Apart from the religious significance it has economical applications and the leaf ,bark, flower and seeds are known to harbour therapeutic properties like its leaves are used to make plates for feasts, wrapping tobacco to make biddies, the bark finds its application as an appetiser, lessening inflammation, treating liver disorders, gonorrhoea ,piles and fractures (Pranshant et al., 2019). This plant is reported to contain valuable phytochemicals such as butrin, butein, terpines, flavonoids and steroids significantly the flower is known to contain coreopsin, isocoreopsin, sulphurein, monospermoside and isomonospermoside (Garima golandaz et.al., 2020). Crucially various parts of Butea monosperma are used in ayurveda and unani demonstratively it can be seen that leaves are used to cure boils, pimples, haemorrhage, kidney disorders and Hemophilia, it is anti-strigent and anti-diabetic, flowers are known for aphrodisiac, anti-inflammatory and anti-cancerous activity, seeds are exclusively used for anti-implantation and anti-ovulatory properties, bark is known for its anti- microbial, anti- fungal, anti- tumor, and anti-ulcerative properties. Butea monosperma a common plant is extensively used in the Ayurveda system (Francis et.al., 2002). The flowers contain flavonoids, saponins, polyphenols which are responsible for anti-oxidant property (Scalbert, Augustin, Ian T. Johnson, 2005).The flowers are used to lower the glucose level in type II diabetes (Alarcon-Aguilar et.al., 2007). Earlier, Butea monosperma has been reported for its various therapeutic potential including antifungal, antimicrobial, antibacterial activity (Garima golandaz et.al., 2020) anti-inflammatory activity (Shahavi and Desai, 2008), liver disorders (Sehrawat et.al.,2006), anticonvulsive activity (, Kasture, and Chopde, 2002 and Kasture, et al, 2000) anti-estrogenic and anti-fertility activity (Patgiri, 2015), heme-agglutinating activity (Ghosh et.al.,1981), wound healing activity (Garima golandaz et.al., 2020) diarrhea (Somani et.al.,2006), giardiasis (Agarwal et.al.,1994), and anthelmintic activity (Iqbal et.al.,2006)

2 Materials and methods

Qualitative Analysis of Butea monosperma

2.1 Preparation of the extract.

Extraction was done by hot continuous extraction (Soxhlet extraction) using methanol as solvent

2.2 Preparation of extract for phytochemical screening (biochemical assay)

80 mg of crude extract was dissolved in 1ml methanol. 10mg (0.010 g) of crude extract is dissolved in ml of methanol).

2.3 Phytochemical screening of the sample (Biochemical assay)

Preliminary screening of phytochemicals in the extracts was performed by biochemical tests specific for secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and cardiac glycosides. The tests performed are as follows. Wagner's test for alkaloids, Molisch's testfor carbohydrates, Killer-killani test for cardiac glycosides, Alkaline reagent test for flavonoids, Ferric chloride test for Phenols, Preupitats test for Phlobatannins, Foams test for saponins, Liebermann Burchard test for steroids, Braymer's test for Tannins, Salkowski test for terpenoids,Test for amino acids and proteins (2 ml of extract was treated with 2-5 drops ninhydrin solution and placed in boiling water bath for 1 -2 min resulting in purple colour solution indicating the presence of amino acid and proteins in the extract.), Tests for Quinones (Few drops of extract when treated with conc HCL the solution turns to yellow precipitate or colouration indicating the presence of quinones.), Tests for Oxalates (3 ml of extract when treated with few drops of ethanoic acid (glacial) if the solution turns into greenish black colour this indicates the presence of oxalates.)Test for fats and fixed oils (When 5 drops of extract treated with 1 ml of 1% CuSo4 solution and 10% NaOH results in the formation of clear solution indicates the presence of fats and fixed oils in the extract.)

2.4 Qualitative analysis of plant sample by HPLC method.

HPLC is an abbreviation for High Performance liquid chromatography. Only compounds dissolved in solvents can be analysed with HPLC. HPLC separates compounds dissolved in a liquid sample and allows qualitative and quantitative analysis of what components and how much of each component are contained in the sample.
The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase eluent and a stationary phase hence different constituents of a sample are eluted at different times there by, the separation of the sample ingredients is achieved.

2.5 Preparation of sample for HPLC analysis.

0.010 g of crude extract is dissolved 1 ml of methanol or Rutin using sonication process for 5 minutes

2.6 Procedure for HPLC analysis for Flavonoids

Filter 1 ml of rutin acid using filter apparatus consisting of silica membrane to remove impurities, transfer the filtered rutin acid in a syringe before that switch on the HPLC instrument to run the mobile phase to release the air gaps present through the tube for 5 minutes. Load 9 drops of rutin acid as a standard and switch on the apparatus allow it to run for 10 minutes, the instrument detects the standard sample and the graph will appear on the system and the values are noted, three trials should be performed. Next the Sample should be filtered through silica membrane to remove impurities and filtered sample is transferred into syringe unload the switch and load 9 drops of sample into the system allow it to run for 10 minutes and the graph will appear on the screen and the values are noted, three trials should be performed.

2.7 Anti-inflammatory activity

Preparation of solutions
0.450 g of Po4 dissolved in 15 ml of distilled water Ph. of 6.3 is maintained, 0006 g of BSA is dissolved in 3 ml distilled water and 0.010 g of the crude extract dissolved in 1 ml distilled water.
The percentage of the inhibition it is calculated by using formula

$$\text{Percentage of inhibition}=\frac{Abs\ of\ c-Abs\ of\ sample\times 100}{Abs\ of\ c}$$

2.8 Anti-cancerous activity

Preparation of solution
0.010 g of the crude extract dissolved in 1 ml of DMSO (Di methyl sulfoxide), prepared 3T3 cell lines extracted from white mice, MTT solution
Procedure for anti-cancerous activity
Anti-cancerous activity of the sample examined using MTT assay (96 wells containing micro titer plate), pipette 300 µl of autoclaved water to border surrounding wells to maintain humidity, add 100 µl of 3T3 cell lines to 6 wells one is kept as control, pipette different 5 concentration of the sample 10 µl 20µl 30µl 40µl 50µl to 5 wells incubate this mixture for CO2 incubation for 48 hours add 100µl of MTT solution to all the wells except water and solution turns purple colour. Results are determined by taking absorbance at 510 nm (optical density) using spectrophotometer.
The percentage of the inhibition it is calculated by using formula

$$\text{Percentage of inhibition}=\frac{Abs\ of\ c-Abs\ of\ sample\times 100}{Abs\ of\ c}$$

3. Results and Discussion

The preliminary analysis conducted showed the result that the compounds like alkaloids carbohydrates, cardiac glucosidase, flavonoids, phenols, phlobatanins, aminoacids and proteins, tannins,quinones, oxalates are presents in the leaf sample but terpenoids, saponins, steroids and fats & fixed oils are absent in the sample (Table 1). The phenolics present in the leaf extract of Butea monosperma are 0.1202 g /g of extract. The flavonoids present in the leaf extract of Butea monosperma are 0.201 g/g of extract.

This study conducted with a aim and hope of finding new therapeutic agents to combat diseases which are largely incurable till now, the plant investigated is Butea Monosperma, collected from the regions of Western Ghats located in Karnataka state of India, this study revealed the following findings, preliminary phytochemical analysis of methanolic leaf extracts revealed the presence of alkaloids, carbohydrates, cardiac glucosidase, flavonoids, phenols, phlobatannins, amino acids and proteins and tannins whereas, saponins, steroids, terpenoids, quinones, oxalates, fats and fixed oils were found to be absent, when compared with the study conducted by Deshmukh et al., (2014) and Malik et al., which showed presence of sterols and triterpenes and glycosides and absence of flavonoids, proteins, alkaloids, and tannins in petroleum ether extract, presence of sterols and triterpenes, glycosides and proteins and absence of flavonoids, alkaloids, and tannins in chloroform extract and presence of flavonoids, proteins and tannins and absence of sterols and triterpenes, glycosides and alkaloids in methanol extract, the small differences may be due to the edaphic, climatic conditions, solvent used in extraction and also due to the difference in age of the plant. Qualitative analysis using HPLC method revealed presence of 0.1202 g of phenols per gram of extract and 0.201 g of flavonoids per grams of extract. Phenols and flavonoids have attracted great attention in relation to their potential for beneficial effects on health over the last few years, several experimental studies have revealed biological and pharmacological properties of these compounds especially their antimicrobial, antioxidant, anti-inflammatory and anti-diabetic potential. The leaves of Butea monosperma are really good for your eyes. When you chew on the leaves or soak them, the liquid that comes out acts as a natural astringent, helps with digestion, and can even work as an appetite booster. It's also helpful for treating coughs, colds, and stomach issues. The liquid can be gargled or swished in your mouth to soothe a sore throat. Plus, it can help manage diabetes and even support the regularity of menstrual cycles in women (Rohith et.al., 2020), leaf extract exhibited significant anti- inflammatory potential and it was agreeably noticed that the activity increased with increase in concentration of the extract used and agreeing reference can be drawn from studies conducted by Choedon et.al., (2010), anti-cancerous activity determined through MTT assay using 3T3 cancer cell linesin leaf extract of Butea monosperma showed positive results and the results are convincingly in agreement with study conducted by Shweta et.al., (2020)

Table 1. Showing Phytochemical tests

Sl SI.No.	Test for compounds	Result
1	Alkoloids	Positive
2	Carbohygrates	Positive
3	Cardiac glucosidase	Positive
4	Flavonoids	Positive
5	Phenols	Positive
6	Phlobatannins	Positive
7	Aminoacids and Proteins	Positive
8	Saponnins	Negative
9	Steroids	Negative
10	Tannins	Positive
11	Terpenoids	Negative
12	Quinones	Negative
13	Oxalates	Negative
14	Fats and fixed oils	Negative

Table 2. Showing Quantitative analysis of Phenols and Falvonoids

SI No.	Compound name	Standard area	Sample area	Gram present in 1g of extract
1	Phenols	14530.7637	17467.2676	0.1202 g

2	Flavonoids	16765.4238	29243.6914	0.201 g

Fig 1. Showing phenols-standard area

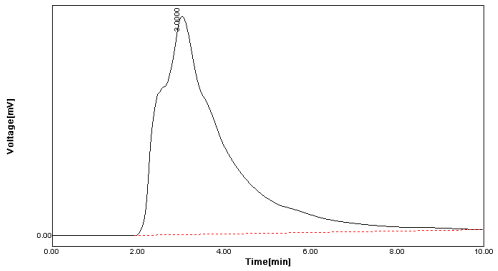


Fig 2. Showing phenols sample area

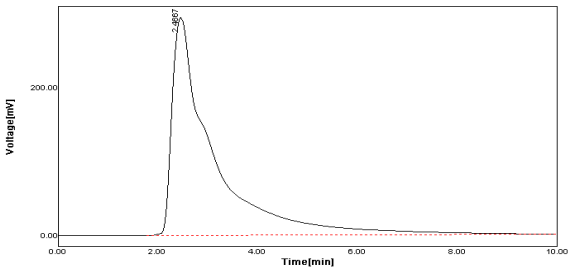


Fig 3. Showing flavonoids standard area

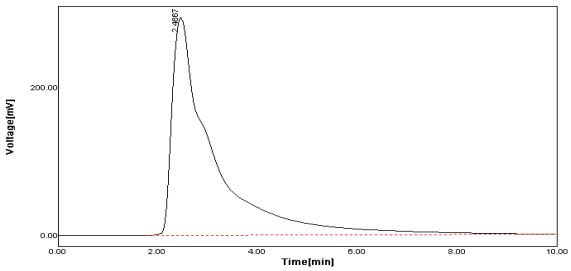


Fig 4. Showing flavonoids sample area

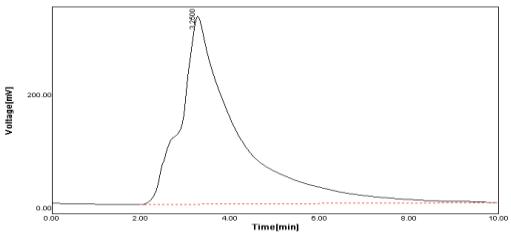
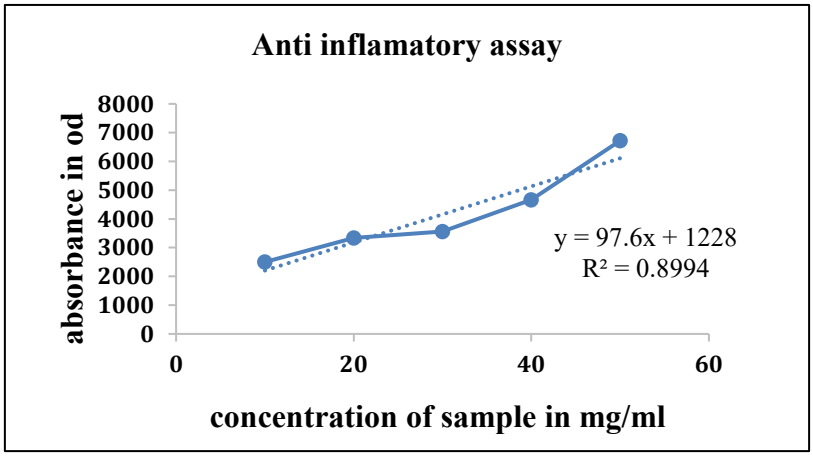


Table 3. Showing Anti-inflammatory activity

Test tube	sample	H_2O	0.1%BSA PH=6.3	Incubate 37^0C for 20 min	Incubate 60^0C for 20 min &cool	Po_4 buffer	OD at 660 nm
B1		50µl					0.120
1	10µl	40µl					0.162
2	20µl	30µl	0.45ml			2.5ml	0.173

3	30µl	20µl					0.228
4	40µl	10µl					0.336
5	50µl						0.005

Fig. 5 Anti-inflammatory activity of sample



Y=97.6x+1228

50=97.6x+1228

97.6x=50-1228

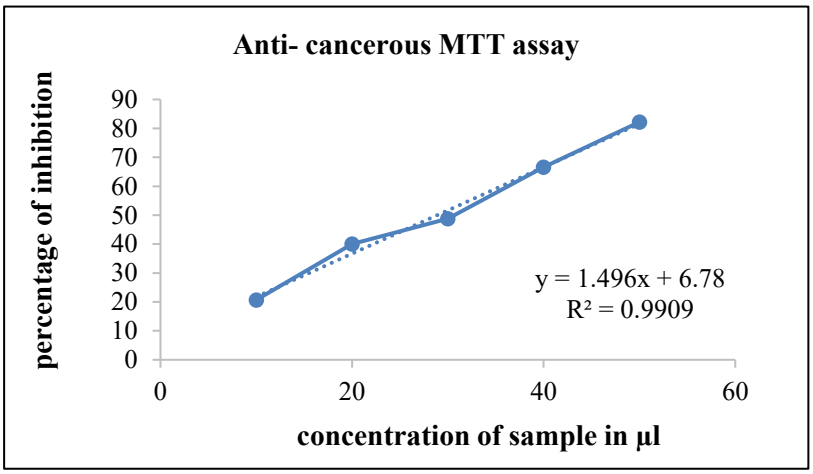
X=-1178÷97.6

X_{50}=-12.06

Table:4 Showing Anticancer activity

Sample in µl	3T3 Cell line	Co_2 incubation for about 48 hours	MTT solution	Co_2 incubation for about 4 hours	DMSO	water	OD at 510 nm
control	100µl to each concentration		50µl for each concentration		200µl to each concentration	800µl to each concentration	0.135
10							0.107
20							0.081
30							0.069
40							0.045
50							0.024

Fig 6. Showing Anti cancerous activity



Y=1.496x+6.78

50=1.496x+6.78

1.496x=50-6.78

X=43.22÷1.496

X_{50}=28.89

4.CONCLUSION

In this study the richness and diversity of metabolites in Butea monosperma is discussed by extracting the secondary metabolites and analyzing its biological activity. Butea monosperma shows anti-cancer activity, which is remarkable because of its metabolite richness and medicinal properties. This study shows a rich concentration of therapeutically important compounds in the extract of Butea monosperma as which can be illustrated by its positive response for anti-cancerous and anti- inflammatory activity. The recent research on the Butea monosperma that adding the flower extract to both the CD and AD groups helped normalize weight gain, showing promise as a herbal remedy for helping obese individuals return to a healthy weight. However, further research is needed to characterize and isolate the compounds individually for their therapeutic properties so that

individual compound can be targeted and efficient drugs can be produced and used in welfare of mankind in various fields like agriculture, pharmaceuticals, environmental protection, industries and economy.

5. REFERENCES

1. Agarwal, A. K., Singh, M., Gupta, N., Saxena, R., Puri, A., Verma, A. K., Saxena, R. P., Dubey, C. B., & Saxena, K. C. (1994). Management of giardiasis by an immuno-modulatory herbal drug Pippali Rasayana. Journal of Ethnopharmacology.44:143-146
2. Alarcón-Aguilar, F. J., Zamilpa, A., Pérez-García, M. D., Almanza-Pérez, J. C., Romero-Nuñez, E., Campos-Sepúlveda, E. A., Vázquez-Carrillo, L. I., & Román-Ramos, R. (2007). Effect of Hibiscus sabdariffa on obesity in MSG mice. Journal of Ethnopharmacology.
3. Chauhan, S., & Mahish, P. (2020). Flavonoids of the flame of forest Butea monosperma. Research Journal of Pharmacy and Technology, 13(11), 5647–5653.
4. Choedon, T., Shukla, S. K., & Kumar, V. (2010). Chemopreventive and anti-cancer properties of the aqueous extract of flowers of Butea monosperma. Journal of Ethnopharmacology, 129(2), 208–213.
5. Deshmukh, M., Sahare, K. N., Patidar, R. K., Mahajan, R., & Singh, V. (2014). Antifilarial activity of Butea monosperma Lam. leaves extracts against Setaria cervi. Herbert Open Access Journals.41-47
6. Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: A review. British Journal of Nutrition. 587–605.
7. Ghosh, B., Dasgupta, B., & Sircar, P. K. (1981). Bacto-Arga—a binding matrix for purification of a lectin from Butea monosperma (Lam) Kuntze. Indian Journal of Biochemistry and Biophysics. 45–52
8. Golandaz, G., Pal, A. K., Uplanchwar, V., & Gautam, R. K. (2020). Butea monosperma flower extract partially reduces high fat diet-induced obesity in experimental rats. Obesity Medicine, 17, 100179.
9. Iqbal, Z., Lateef, M., Jabbar, A., Ghayur, M. N., & Gilani, A. H. (2006). In vivo anthelmintic activity of Butea monosperma against trichostrongylid nematodes in sheep. Fitoterapia, 77(2), 137–140.
10. Jangid, M., Chauhan, P., Neetha, P., Chopra, R., Nagar, B., Aravindakshan, K., & Nagar, A. (2021). Studies on phytochemical screening of flowers and chlorophyll analysis of Butea monosperma (Lam.) Kuntze, Jhalawar. International Journal of Current Microbiology and Applied Sciences, 10, 3371–3375
11. Kasture, V. S., Chopde, C. T., & Deshmukh, V. K. (2000). Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosa-sinensis, and Butea monosperma in experimental animals. Journal of Ethnopharmacology.
12. Kasture, V. S., Kasture, S. B., & Chopde, C. T. (2002). Anticonvulsive activity of Butea monosperma flowers in laboratory animals. Pharmacology Biochemistry and Behavior, 72(4), 965–972
13. Malik, N. Z., Mazher, M., Riaz, M., Ahmad, B., & Noshad, Q. Q. (2014). Morphology, phytochemistry and antifungal activity of Butea monosperma Lam. Pakistan Journal of Botany, pages 1–9
14. Patel, G., Pandey, P., Tripathi, I. P., & Singh, R. (2019). Estimation of antidiabetic, antioxidant and phytochemical constituents of two species of Butea monosperma (Palash). Indian Journal of Applied Research. 57–59.
15. Patel, G., Dwivedi, N., & Tripathi, I. P. (2017). Biochemical studies of Butea monosperma (Palash). International Journal of Current Research, 9(2), 45965–45968.
16. Rohit, Y. S., Sonali, S., Kumar, P. A., & Shubham, P. S. (2020). Butea monosperma (Palash): Plant review with their phytoconstituents and pharmacological applications. IOSR Journal of Pharmacy and Biological Sciences, 15, 18–23.
17. Sehrawat, A., & Sultana, S. (2006). Chemoprevention by Butea monosperma of hepatic carcinogenesis and oxidative damage in male Wistar rats. Asian Pacific Journal of Cancer Prevention, 7, 140–148.
18. Shahavi, V., & Desai, S. K. (2008). Anti-inflammatory activity of Butea monosperma flowers. Fitoterapia, 79, 82–85.
19. Somani, R., Sanjay, K., & Abhay, K. S. (2006). Antidiabetic potential of Butea monosperma in rats. Fitoterapia, 77, 86–90.
20. Tiwari, P., Jena, S., & Sahu, P. (2019). Butea monosperma: Phytochemistry and pharmacology. [Journal/Publisher details missing], 3, 19–26.
21. Yadav, S., Patgiri, B., & Prajapati, P. (2015). Review of bio-active principle of Butea monosperma (Lam.) Kuntze. Indian Journal of Scientific Research, 1, 45–51.