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Estimation of Total Phenolic, Flavonoid and Saponin Content in Different Extracts of *Butea monosperma* Bark.

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ABSTRACT
The Indian flora is extensively utilized as source of drugs mentioned in the traditional systems of medicine. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of India. *Butea monosperma* (palas) belonging to the family fabaceae is grown widely in many parts of India. The plant is highly used by the rural and tribal people in curing various disorders. The present work was aimed to estimate the total phenolic, flavonoid and saponin contents in ethyl acetate and methanol extracts of *Butea monosperma* bark. The total phenolic content was determined by folin-ciocatechu reagent using gallic acid as standard and total flavonoid content by aluminium chloride assay using rutin as a standard .The total saponin content was determined by vanillin reagent using diosgenin as a standard. Ethyl acetate and methanol extracts of *Butea monosperma* bark have a significant amount of secondary metabolites like phenolic compounds, flavonoids and saponins. Methanol extract of bark showed highest amount of total phenolic content (295.83±0.57 mg/g equivalent of Gallic acid) and total flavonoid content (372.55±0.50 mg/g equivalent of rutin). Ethyl acetate extract of bark showed highest amount of total saponin content (465.66±0.57 mg/g equivalent of diosgenin). The presence of such important secondary metabolites in *Butea monosperma* bark indicates its therapeutic importance in man and animal.

KEYWORDS: *Butea monosperma***,** bark, phenolic, flavonoid, saponin, extract.

INTRODUCTION
Natural products have played an important role throughout the world in treating and preventing human diseases. Plant-derived substances are of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicines; therefore, any scientific data on such plant derivatives could be of clinical importance [2].The major merits of herbal medicines seem to be their efficacy, low incidence of side effects and low cost [3].

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [4]. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently in vogue in parts of the world [5]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region [6].

Butea monosperma (Lam.) Taub is commonly known as 'Flame of forest', belongs to the family Fabaceae. It is locally called as Palash, Palas, Mutthuga, Bijasneha, , Khakara, Dhak Chichra, Bastard Teak, Bengal Kino, Nourouc and is common throughout India, Burma and Ceylon except in very acrid parts [7][8][9]. Almost all

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the parts of plant including flowers, seeds, leaves and barks possess medicinal property [10].The plant holds a significant place because of its medicinal and other miscellaneous uses of economic value. The *Butea monosperma* bark contain Kinotannic acid, Gallic acid, pyrocatechin, butrin, palasitrin, alanind, allophonic acid, butolic acid, cynidin, histidine, lupenone, lupeol, miroestrol, palasimide and shelloic acid [7]. The plant is regularly used by the rural and tribal people in curing various disorders [11]. The aim of the present study was to quantitatively estimate the total phenolic, flavonoid and saponin contents in ethyl acetate and methanol extracts of *Butea monosperma* bark.

MATERIALS AND METHODS

Collection of plant material

The bark of plant was collected from the nearby area of Bhopal, Madhya Pradesh, India. The plant was identified and authenticated by Dr. Zia Ul Hasan, Professor& Head -Department of Botany, Safia college of Science, Bhopal.

Preparation of plant extracts

The plant material was shade dried for about 15 days. The dried sample was then coarse powdered and stored in a sterile container. The successive extraction of the samples from non polar to polar solvents was done by using three different types of solvents such as petroleum ether, ethyl acetate and methanol using standard technique of maceration. The extracts thus obtained were evaporated to dryness at room temperature and stored in a sterile air tight container. The concentrated mass obtained, i.e. the crude extract for the three solvents was weighed and kept in a refrigerator for further experimental procedure.

Estimation of Total Phenolic Content [12] [13]

The total phenolic content of the *Butea monosperma* bark extracts were determined by using Folin-Ciocalteu reagent. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The total phenolic contents were expressed as mg/g gallic acid equivalent.

Estimation of Total Flavonoid Content [14]

The total flavonoid content of *Butea monosperma* bark was determined using the aluminium chloride assay. An aliquot (0.5 ml) of extracts were taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% NaNO2, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminium trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used as blank. The total flavonoid content was expressed in mg of rutin equivalents per gram of extract.

Estimation of Total Saponin Content [15]

Standard saponin solution was prepared by dissolving 10 mg of diosgenin and add (16 ml) methanol and distilled water (4 ml). To the aliquots for each tube, vanillin reagent (8%, 0.25 ml) was added and sulphuric acid (72% v/v, 2.5 ml) added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60 ^{0}C water bath. After 10 mins incubation, the tubes were cooled in ice cold water bath for 3 – 4 min. The absorbance was measured at 544 nm against the reagent blank. 0.1 g of freeze dried sample was dissolved in aqueous methanol (80%, 0.1 ml). 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm.

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RESULTS AND DISCUSSION

Table 1: Total phenolic, flavonoid and saponin content of *Butea monosperma* bark extracts.

S.No.	Phytochemicals	Ethyl acetate extract	Methanol extract
1.	Total phenolic content in mg/g equivalent of Gallic acid	160.16±0.28	295.83±0.57
2.	Total flavonoid content in mg/g equivalent of Rutin	116.73±0.23	372.55±0.50
3.	Total saponin content in mg/g equivalent of Diosgenin	465.66±0.57	269.33±0.57

The values are means of three replicates with standard deviations (mean ± S.D)

Figure 1: Standard curve of Gallic acid for estimation of Total Phenolic Content.

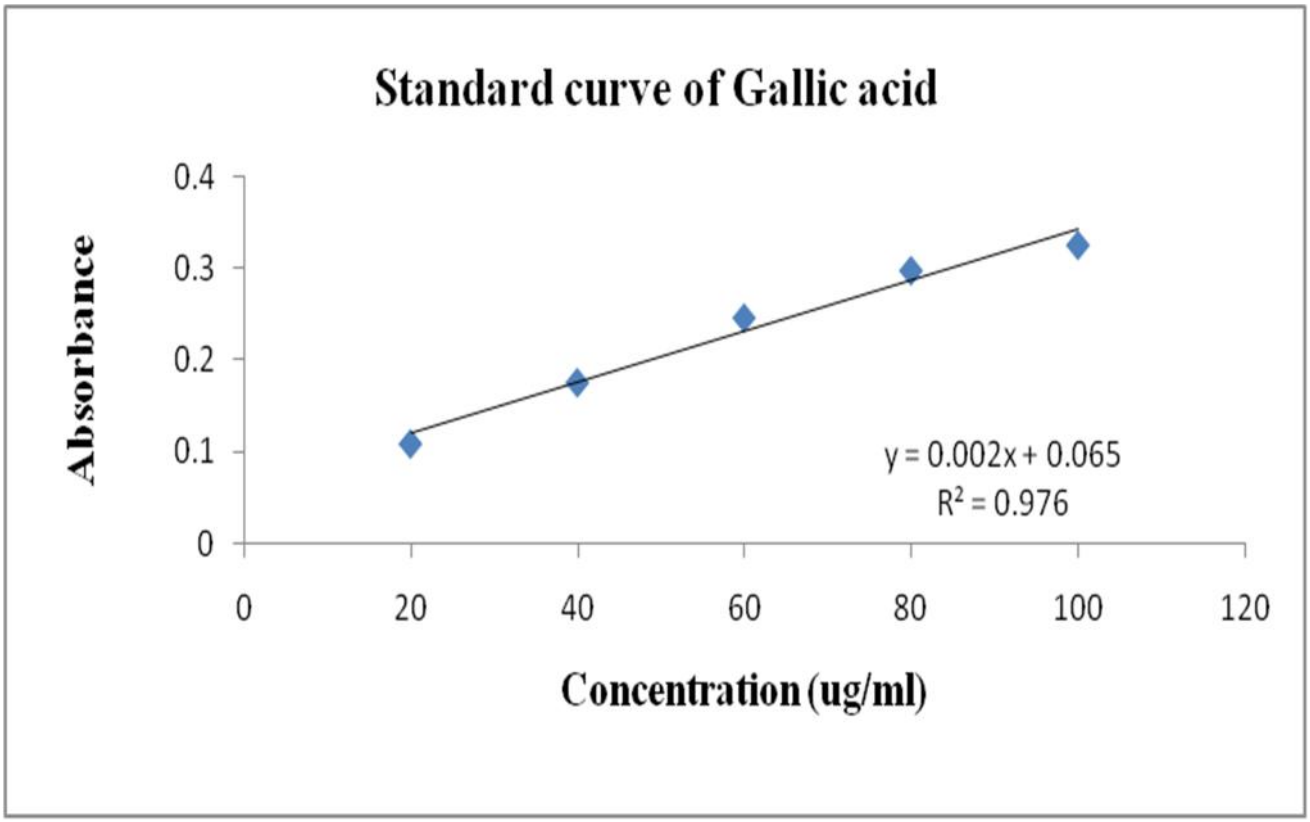

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Figure 2: Standard curve of Rutin for estimation of Total Flavonoid Content.

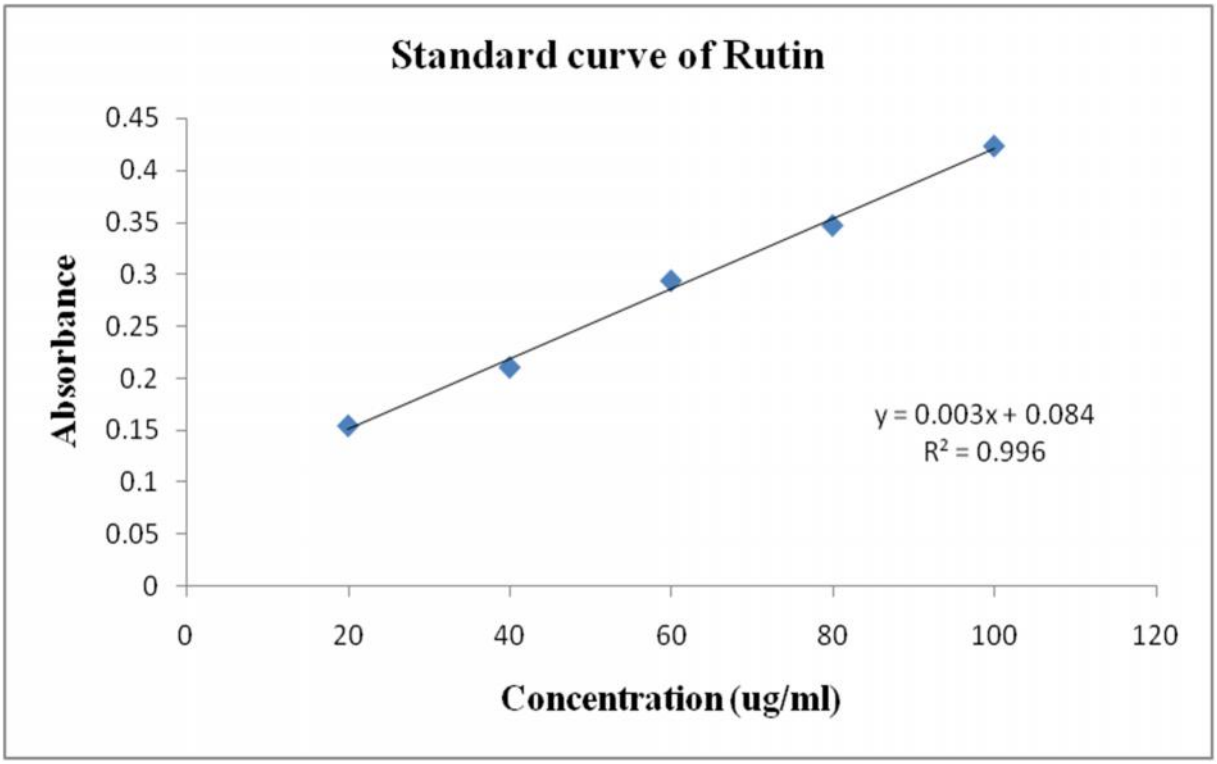
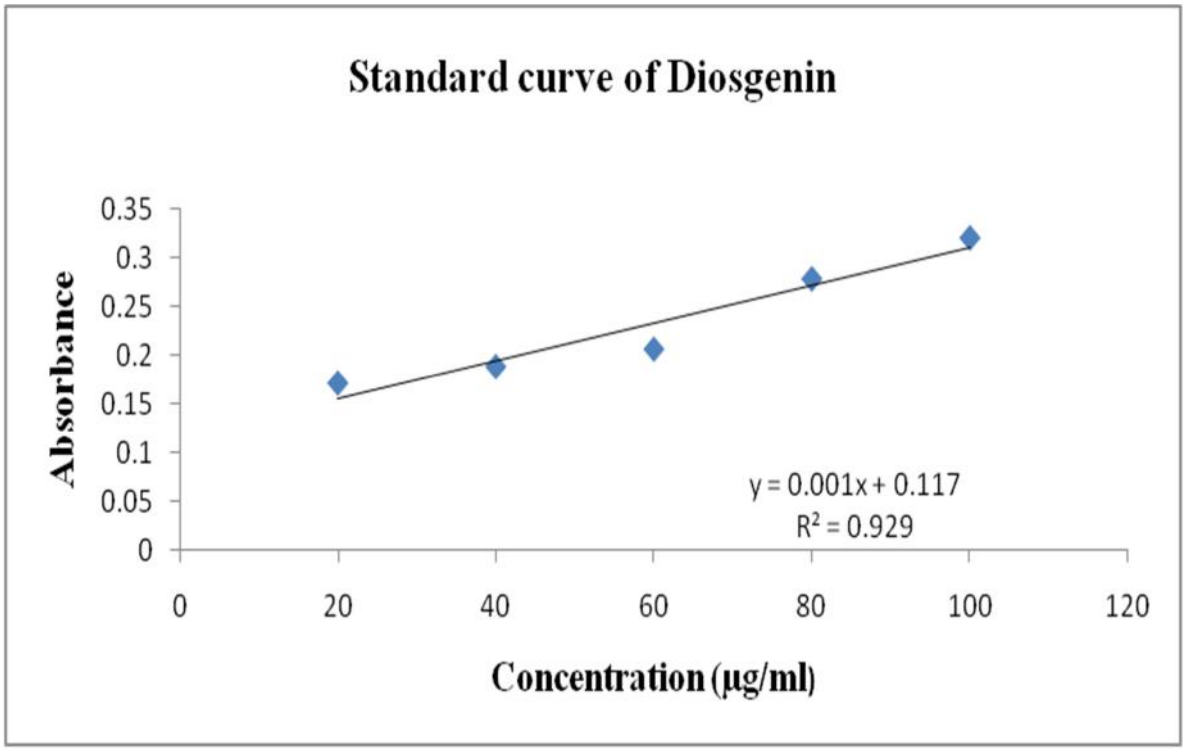


Figure 3: Standard curve of Diosgenin for estimation of Total Saponin Content.



Medicinal plants possess great potential uses, especially as traditional medicines. Knowledge of the chemical constituents of medicinal plants is helpful in the discovery of therapeutic agents as well as new sources of new drug candidates. The biological efficacy of these plants in turn depends on the presence of the required quantity and nature of the secondary metabolite in the crude extract [16]. In the present study quantitative estimation of the total phenolic, flavonoid and saponin contents in ethyl acetate and methanol extracts of *Butea monosperma* bark was done.

The total phenolic content was determined using Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenolic contents were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.002x + 0.065$, $R^2 = 0.976$(Figure 1); where y is absorbance at 765 nm and x is total phenolic content in the extracts of *Butea monosperma* bark expressed in mg/g equivalent of Gallic acid. The total phenolic content in the ethyl acetate and methanol extract of *Butea monosperma* bark was found to be 160.16±0.28 and 295.83±0.57 in mg/g equivalent of Gallic acid (Table 1). The total flavonoid content was determined using aluminum tri chloride reagent. Rutin was used as a standard compound and the total flavonoid content was expressed as mg/g rutin equivalent using the standard curve equation: $y = 0.003x + 0.084$, $R^2 = 0.996$ (Figure 2); where y is absorbance at 510 nm and x is total flavonoid content in the extracts of *Butea monosperma* bark expressed in mg/g. The total flavonoid content was 116.73±0.23 and 372.55±0.50 in mg/g equivalent of rutin in the ethyl acetate and methanol extracts, respectively (Table 1). The total saponin content was determined using vanillin reagent. Diosgenin was used as a standard compound and the total saponin content were expressed as mg/g diosgenin equivalent using the standard curve equation: $y = 0.001x + 0.117$, $R^2 = 0.929$(Figure 3); where y is absorbance at 544 nm and x is total saponin content in the extracts of *Butea monosperma* bark expressed in mg/g. The total saponin content in the ethyl acetate and methanol extract of *Butea monosperma* bark was found to be 465.66±0.57 and 269.33±0.57 in mg/g equivalent of diosgenin (Table 1). Methanolic extract of bark showed highest amount of total phenolic content (295.83±0.57 mg/g equivalent of Gallic acid) and total flavonoid content (372.55±0.50 mg/g equivalent of rutin). Ethyl acetate extract of bark showed highest amount of total saponin content (465.66±0.57 mg/g equivalent of diosgenin). The presence of such important secondary metabolites in *Butea monosperma* bark indicates its therapeutic importance in man and animal.

CONCLUSION

From the current study, we can conclude that ethyl acetate and methanol extracts of *Butea monosperma* bark have a significant amount of secondary metabolites like phenolic compounds, flavonoids and saponins. However further investigations are required to isolate and characterize the active constituents from this plant to evaluate their therapeutic role.

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