International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 3, 2011

Research Article

PHYTOCHEMICAL SCREENING, ANTIBACTERIAL ACTIVITY AND PHYSICOCHEMICAL EVALUATION OF LEAVES OF *BUTEA MONOSPERMA*

AMBERSING RAJPUT [1], SUBOTH CHANDRA PAL [2], BHAGAVAN PATIL [3]

[1]Department of Chemistry Z.B.Patil College, Dhule Maharashtra, 424002, [2] N.D.M.V.P. College of Pharmacy, Nasik, Maharashtra,-409429, [3]G.M.C.Polytechnic, Shahada, Dist Nandurbar, Maharashtra-425409, India. Email: bhagavanpatil1@rediffmail.com

Received: 07 March 2011, Revised and Accepted: 06 April 2011

ABSTRACT

The purpose of present work is to study medicinally active substances present in solvent-extracts obtained from leaves of *Butea monosprema*. The active substances were isolated by Soxhlet extractor and identified by phytochemical test. The Soxhlet extraction of leaves, in powdered form, was performed using petroleum ether and followed by chloroform and methanol. The results of analyses of each extract confirm the active substance were sterols, triterpenes, glycosides flavonoids and proteins. The evaluation of leaves powder was supported by physico-chemical analysis. The microbial tests of isolated substances were performed with microorganisms like *Escherichia coli, Pseudomons aeruginosa, Bacillus subailis, Staphylococcus aureus, Proteus vulgaris* and *Klebshiella pneumonia*. The observation of microbial test of methanol-extract supports to antibacterial activity to the greater extent than petroleum ether and chloroform-extract.

Keywords: *Butea monosperma*, Phytochemical test, Antibacterial activity & Physico-chemical constant.

INTRODUCTION

The *Butea monosperma* belongs to family fabeacae. It is a medium sized tree with 20-40 feet height and found in mountain region of India, Burma and few Asian countries[1]. In literature, the *Butea monosperma* is known for several medicinal properties. The flowers are widely used in treatment of hepatic disorders, viral hepatitis, and diarrhea[2]. The flowers have anticonvulsive, antihepatotoxic, anti-implantation, hypoglycemic, astringent, diuretic aphrodisiac properties and tonic[3-4]. The flowers are good source of flavonoids. The contents of flowers are butein, butrin, isobutrin, plastron, coreipsin and isocoreipsin[5-6]. The roots are useful in treatment of night blindness, filariasis, piles, ulcers and tumors [7]. The stem bark posses antifungal activity. The compounds isolated from stem bark are stigmasterol, stigmasterol-βD-glucopyranoside, nonacosanoic acid, 3 α- hydxoxyeuph-25-ene and 2,14-dihydroxy-11, 12-dimethyl-8-oxo-octadec-11- encyclohexane[8]. The gum is powerful astringent. The literature survey revealed that less work is done on leaves of *Butea monosperma.*

The present work is a study about preliminary phytochemical, antibacterial activity of three solvent extracts and physicochemical analysis of leaves of *Butea monosprema* were conducted. The *Butea monosperma* leaves were collected in the summer season of year 2007 from land which near to Village-Mhasawad, District-Nandurbar Maharashtra, India. The land of this village is part of famous Satpuda ranges. The plant specimen were identified and authentificated from Dr. S.K.Tayade, Taxonomist, Department of Botany, Arts, Commerce and Science College of Shahada. The authentification was confirmed, additionally, from Botanical Survey of India Pune.

The leaves were shade dried and milled to get powder using mechanical grinder. The sieved powder was used for evaluation and extraction purpose. The physico-chemical analysis was performed on leaves powder. The physicochemical analysis includes ash content, water soluble and alcohol soluble extractive[9-12]. The results of physico-chemical test were tabulated in Table-1.

Table1: Physico-chemical analysis

Sr. No.	Parameters	Result (%w/w)
1	Total ash	9.25
2	Acid insoluble ash[1]	9.01
3	Water soluble ash[1]	0.75
4	Water soluble Extractive	10.8
5	Alcohol soluble Extractive	7.2

[1]Insoluble ash of total Ash

The powder of *Butea monosperma leaves* was extracted by Soxhlet apparatus using solvent petroleum ether (60-80^{0}C) and followed by chloroform and methanol[13]. Accurately weighed 50 gm of powder was placed in Soxhlet extractor. About 750 ml of solvent was used for extraction. The progress of the extraction was evaluated by applying spot of extract on thin layer chromatography plate. The thin layer chromatography was performed using silica gel plate and the plate was visualized in UV-chamber followed by iodine chamber. The extracts were filtered and concentrated by rotary evaporator and finally dried at very low pressure. The phytochemical test was performed for each extract[14-18]. The results of phytochemical tests were tabulated in Table-2.

The concentrated extracts were filtered using Whatman filter paper No.1. The filtrate was evaporated under reduced pressure and dried using rotary evaporator at about 55^{0}C. The dried extracts were preserved in labeled sterile screw capped bottles at –20 ^{0}C. The test solution of extracts and standard solution were prepared. The concentration of extract was set to 0.1 mg/ml in dimethylsulphoxide. The drug used in standard preparation was chloramphenicol of IP grade. The antibacterial activity was performed using 24 hours cultures of *E. coli, P. aeruginosa, B. subtilis, S. aureus ,P. vulgaris* and *K. pneumonia* developed in Muller Hinton agar medium[19-23]. The bacterial strains were used and obtained from NCIM, Pune. The aliquot of 1ml quantity of test and standard solution was transferred in 6 mm well. The stringent aseptic conditions were maintained during microorganism inoculation and the plates were labeled. The test and standard solution were allowed to diffuse in wells for 2 hours at room temperature. The Petri plates were incubated at 37±1^{0}C for 24 hours. The diameter of zone of inhibition of each well was recorded. The results of antibacterial activity were tabulated in Table-3.

Table 2: Phytochemical screening

Sr. No.	Compound	Test	Extracts		
			Petroleum ether	Chloroform	Methanol
1	Sterols and Triterpenes	Libermann-Buchards test	+	+	-
		Salkwaski test	+	+	-
2	Glycosides	Borntragers test	-	+	-
		Modified Borntragers test	-	+	-
3	Flavonoids	Shinoda test	-	-	+
		Lead acetate test	-	-	+
4	Proteins	Biuret test	-	+	+
		Millons test	-	+	+
5	Alkaloids	Wagner test	-	-	-
6	Tannin	5%$FeCl_3$ Solution	-	-	+

(+) meets the test requirement qualitatively and (-) do not meets the test requirement qualitatively

Table 3: Antibacterial screening

Extract	Zone of inhibition (mm)					
	E. Coli	*P. aeruginousa*	*B. substalis*	*S. aureus*	*P. vulgaris*	*K. Pneumonia*
Control (DMSO)	-	-	-	-	-	-
Petroleum Ether	7.5	8.3	11.5	17.5	15.0	7.8
Chloroform	-	7.8	9.2	4.2	14.3	7.9
Methanol	-	14.3	7.3	15.3	9.2	15.8
Chroam- Phenicol	30.1	25.2	30.1	33.1	30.2	29.5

(–) means no zone of inhibition,[1]Concentration of Chloramphenicol was set to10µg/well

It was clear from the experimental data presented in Table-1 that the quantitative ash content value suggest the presence of inorganic component in leaves *Butea monosperma*. The quantitative solubility of ash was checked in dilute acid solution and water. The solubility of ash in dilute acid solution was found lower than water. The water soluble and alcohol soluble extractive were checked quantitatively. The water soluble extractives were found higher than alcohol soluble extractives. The data obtained from physicochemical test was used to evaluate the possibility of adulteration of quality of leaves. It was clear from the experimental data presented in Table-2 that the substances like sterols, triterpenes, glycosides flavonoids and proteins were medicinally active components of leaves of *Butea monosperma*. The petroleum ether extract confirms the presence of sterols and triterpenes. The chloroform extract confirms the presence of sterols, triterpenes as glycosides. The methanol extract confirms the presence of flavonoids and proteins. It was clear from the experimental data presented in Table-3 that antibacterial activity was showed by each extract against all the strains. The methanol and petroleum ether extract showed significant antibacterial activity as compared to chloroform extract. The lowest inhibitory activity was recorded for *E.coli.* The maximum inhibition zone was observed for *S.aureus* followed by *P.aeruginosa* & *K.pneumonia* and *B.subtalis*.

ACKNOWLEDGMENT

The authors would like to thanks to Prof. M.N.Patil, Co-ordinator, P.S.G.V.P.M's education institute, Dr. A.S.Patil, Principal, Arts, Commerce and Science College and Dr. S.P.Pawar, Principal, Pharmacy College based at Shahada for their valuable support and permission to use laboratory facilities.

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