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ANTIDEPRESSANT POTENTIAL OF ISOLATED BIOACTIVE COMPOUND FROM *BUTEA MONOSPERMA* (LAM.) KUNTZE

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Abstract

Traditional or alternative medicine refers other than orthodox medicine. From time immemorial, plants have been utilized as corrective agents for a variety of diseases. Butea monosperma (Lam.) Kuntze is a generally utilized plant in Ayurveda. Butea monosperma (Palas) has a place with the family Fabaceae, developed freely in many parts of India. Different phytochemical

constituents present are carbohydrates, alkaloids, tannins, flavonoids, steroids and terpenoids. Butea monosperma is traditionally utilized as a free radical scavenger, antistress, CNS stimulant, antigout, diuretic, antileprotic, anti-inflammatory, antiulcer, astringent and antihepatotoxic. Its flower is utilized to treat instances of expanded spleen, menstrual aggravations, consuming sensations and eye diseases.

Effect of Isolated bioactive compound from Methanol Extract of Butea monosperma (Lam.) Kuntze (BMME) was studied for the duration of immobility by Forced Swim Test (FST) At the dose of 20 mg/kg isolated compound showed significant effect ($p<0.05$) for duration of immobility when compared with control group of animals.

The monoamines are determined in mice brain to find out the altered levels of monoamines. It had been suggested that an increase in both swimming and climbing behaviors in the FST occurs when the animal is treated by a drug which increases NA and DA levels in the nerve terminals. An increase in NA and DA could be by inhibition of MAO activity in the brain.

Keywords

Ayurvedic Medicine, *Butea Monosperma*, Antidepressant, Forced Swim Test, Acetylcholinesterase, Monoamine

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1. Introduction

Ayurveda is Indian ancient medical system primarily practiced and has been known from 5000 years. Ayurveda emphasize the body, mind and spirit in disease treatment and prevention while it includes herbal remedies and diet (Morgan, 2002). Use of CAM has increased and the utilization pattern analyzed by various statistical data analysis show very encouraging and promising results (Viji and Helen, 2011).The market value of herbal medicines currently stands at over $ 60 billion per anum globally. The sale of herbal medicines is growing at annual growth rate of 6.4% (Inamdar et al., 2008).

The drugs that are commonly prescribed for treatment of depression include selective serotonin reuptake inhibitors like fluoxetine, nor-adrenaline reuptake inhibitors like amitriptyline, and monoamine oxidase inhibitors like selegiline and atypical antidepressants like trazodone. A number of drugs are available for the treatment of depression, but clinical monitoring of these drugs have shown incidence of adverse effects, relapses and drug

interactions (Ray et al., 1987; Roose et al., 1994). MAO inhibitors can also initiate sedation or behavioral excitation and have a high risk of inciting postural hypotension, once in a while with sustained little rises of diastolic blood pressure. SSRIs have a high risk of nausea and vomiting, sexual dysfunction including impaired orgasm in women, inhibited ejaculation in men and and headache. Some SSRIs like fluoxetine in particular, cause agitation and restlessness that resembles akathisia (Hamilton and Opler, 1992).

Herbal medicines are now extensively used due to their wide applicability, acceptability and therapeutic efficacy with least adverse effects, which in turn has accelerated the progress of scientific research regarding the new development of herbal antidepressant drugs. Because of the limited availability of the antidepressant drugs, attempts are going on to explore plants with antidepressant potential. Chemical substances obtained from plant sources have been used to treat various human diseases from the ancient times. Recent technological advancements have renewed interest in natural products for new drug discovery.

2. Isolation of Compound From The Methanol Extract of Stem of *B. monosperma*

The methanol extract of stem of *B. monosperma* (100 g) was dissolved in methanol and centrifuged at 2000 rpm for 30 min. The suspension was added to silica gel and evaporated to dryness. The residue was placed on top of the silica gel column (60-120 mesh) and eluted with chloroform: methanol (95:05 v/v). The fraction eluted with chloroform: methanol (95:05 v/v) was further re-chromatographed by column chromatography (on silica gel 100-200 mesh). A pure compound was eluted by chloroform: methanol (50:50, v/v) (Gomes *et al*., 2006).

The trivial name TBM was given for convenience. TLC study of the isolated compound TBM has been tabulated below.

Table 1: *TLC study of isolated compound TBM*

Name of Isolated Compound	% Yield	Solvent system	R_f Value	Inference

TBM	0.29% $^{w}/_{w}$ with respect to crude powder of *B. monosperma* on dry wt basis	Benzene: ethyl acetate (70:30, $^{v}/_{v}$).	0.52	A single R_f value indicates a single compound may be isolated

In TLC the single spot at R_f 0.52 clearly showed the presence of a single compound which was isolated from BMME by chromatographic method. The % yield of the isolated compound was calculated as 0.29% $^{w}/_{w}$. In this isolation process mixture of benzene: ethyl acetate (70:30, $^{v}/_{v}$) was used as the mobile phase.

Preliminary phytochemical analysis had given positive test for triterpenoid by all methods and negative test for alkaloid, carbohydrate, tannin, phytosterol, saponin and flavonoid. The results obtained indicated the strong presence of triterpenes and this confirmed the isolated compound obtained from BMME was triterpene in nature.

3. Preliminary Phytochemical Screening of The Isolated Compound From BMME

3.1 Test for Triterpenoids

Salkowski Test: To a test tube 0.2 g extract was mixed with 2 mL of chloroform and filtered. The filtrate was treated with few drops of concentrated H_2SO_4, shaken and allowed to stand. Appearance of golden yellow colour showed positive result for the presence of terpenoid.

4. Spectral Studies

The characterization of isolated compound was carried out by FTIR, ^{1}HNMR, ^{13}CNMR and MASS spectral studies.

FTIR spectrum was recorded with KBr pelletes on a Perkin-Elmer 1710 FTIR spectrophotometer.

^{1}HNMR and ^{13}CNMR spectra were obtained by Agilent VNMRS spectrophotometer at 27°C. $CDCl_3$ was used as solvent in both ^{1}HNMR and ^{13}CNMR.

The MASS spectrum was recorded on a spectrophotometer (Bruker micrOTOF-Q II 10262 ESI). Source type was ESI, ion polarity was positive, dry heater was set at 180°C and dry gas was set at 4.0 L/min. The software used was Bruker Compass Data Analysis 4.0.

5. Antidepressant Activity

5.1 Forced Swim Test (FST)

Behavioural despair was proposed as a model to check for antidepressant potency. It was suggested that mice or rats when put in a restricted space from which they cannot escape, are forced to swim and induced to a characteristic behaviour of immobility.

Swimming sessions were conducted by placing animals inside individual glass cylinders (45 cm high × 20 cm in diameter) containing (25±2°C) water 38 cm deep, so animals were not able to support themselves by touching the bottom with their feet or tail. Two swimming sessions were performed between 12:00 h and 19:00 h. Doses were given once daily for 7 days. On the 7^{th} day animals were subjected to 15 min pre-test. After 15 min spending in the water the animals were removed and allowed to dry in a warm closed area (32°C) before being returned to their home cages. They were again placed in the cylinder on the 8^{th} day, 24 h after pretest and the total duration of immobility was measured during a 6 min test. Floating behaviour during this 6 min period had been found to be reproducible in different groups of rats. An animal was marked to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The mouse was considered immobile when it floated motionlessly or made only those minimum movements necessary to keep its head above the water surface. The total immobility time for the period of 6 min was recorded with the help of stopwatch (Porsolt *et al*., 1977 and 1978).

5.2 Materials and Method

Fluoxetine (Sigma Aldrich, Bangaluru) and CMC (CDH, Mumbai) were used for the study.

The experimental animals were divided into five groups containing nine animals each and depression was induced in all animals (except negative control) by FST. Treatment was given by administering fluoxetine and isolated compound at a dose of 20 mg/kg bw i.p. and the animals were treated as follows :

Group 1 (Negative Control): Normal animals received 1%w/v CMC in distilled water at a dose

of 1 mL/kg bw p.o.

Group 2 (Positive Control): Depression induced animals received 1%w/v CMC in distilled water 1 ml/kg bw p.o.

Group 3 (Standard): Depression induced animals received fluoxetine suspended with 1% w/v CMC in distilled water and administered at a dose of 20 mg/kg bw p.o.

Group 4 (Isolated Compound): Depression induced animals received the isolated compound TBM from BMME suspended with 1% w/v CMC in distilled water and administered at a dose of 20 mg/kg bw p.o.

6. Procedure for Estimation of MAO- A

Adult Swiss albino mice (30-35 g bw) were used for the experiment. Animals were housed in groups of 3 for every cage and kept up in the animal house. The animals were kept up in the animal house according to the rules of CPCSEA. All endeavors were made to minimize both the quantity of animal utilized and undesirable anxiety or inconvenience to the animals amid trial strategies. All the samples taken were done in the between of 07:00 and 09:00 AM with a specific end goal to maintain a strategic distance from circadian rhythm initiated variation. The mice were sacrificed; brains were taken out rapidly under chilled condition and put in ice cold saline.

250 µL of the whole brain homogenate was added to 250 µL of serotonin and 250 µL of buffer. The reaction tube was placed at 37°C for 20 min and the reaction was arrested by the addition of 200 µL of 1M HCl. The reaction product was extracted with 5 mL of Butyl acetate. The organic phase was separated and measured at 280 nm using spectrophotometer. Blank samples were prepared by adding 1M HCl (200 µL) prior to reaction and the reaction was carried out. The MAO-A is expressed in nmoles/mg protein (Pal, 2009).

7. Statistical Analysis

Data were expressed as mean ± Standard Error Mean (SEM). Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test group vs control group. For numerical results, one-way analysis of variance (ANOVA) with Dunnett's 't' test (compare all groups vs. control group) was performed using Prism Graph Pad InStat Version 5.00 (Prism GraphPad Software).

8. Results and Discussion

8.1 Spectral Analysis

FTIR spectroscopic analysis of isolated compound TBM from the methanol extracts of stem of *Butea monosperma* (Lam.) Kuntze

The FTIR spectra was interpreted as follows

IR (KBr): 3384.58 cm-1(Hydrogen bonded OH Stretch), 2942.36 cm^{-1} (C-H Stretch in CH_2), 1645.85 cm-1 (C=C Symmetric Stretch), 1485.69 (C=C Asymmetric stretch), 1463.19 cm-1(C-H deformation in CH_2 and CH_3), 1388.10 cm-1(C-H Stretch), 1027.79 cm-1(C-O Stretch of secondary alcohol), 882.87 cm-1(=C-H bending exocyclic CH_2) (Haque *et al.*, 2008).

NMR spectral study of isolated compound TBM from the methanol extract of stem of *Butea monosperma* (Lam.) Kuntze

^{1}HNMR of isolated compound TBM from the methanol stem extract of B. monosperma

The ^{1}HNMR: 7.260 ($CDCl_3$ peak), 4.683, 4.565(H-29, d,d, 2H), 3.202-3.194 (H,3, d,d, 1H, 6 Hz, 5Hz), 2.392(H-19, m, 1H), 2.381 (H-21a, m, 1H), 2.360 (H-15A, t, 1H), 2.348 (H-30, s, 3H), 1.609 (H-12A, 1A, d, 2H), 1.458 (H-13, t, 1H), 1.322 (H-2A, d, 1H), 1.232 (H-2B, q, 1H), 1.198 (H-12A, q, 1H), 1.176 (H-23, s, 3H), 1.080 (H-15A, d, 1H), 0.966 (H-23,s, 3H), 0.942 (H-27, s, 3H), 0.905 (H-18, t, 6 Hz, 1H), 0.787 (H-28, s, 3H),0.759 (H-24, s, 3H), 0.690 (H-25, s, 3H), 0.671 (H-5, d, 1H) (Haque *et al.*, 2008).

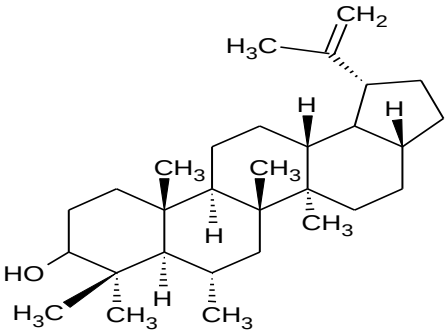
^{13}CNMR of isolated compound from the methanol extract of stem of B. monosperma

In the ^{13}C NMR spectrum of Lupeol showed δC: δ 37.354 (C-1), δ 21.108 (C-2), δ 79.178 (C-3), δ 38.241 (C-4), δ 55.481 (C-5), δ 18.171 (C-6), δ 27.626 (C-7), δ 38.886 (C-8), δ 50.625 (C-9), δ 34.463 (C-10), δ 19.476 (C-11), δ 21.108 (C-12), δ 35.760 (C-13), δ 39.031 (C-14), δ 25.334 (C-15), δ 29.857 (C-16), δ 40.176 (C-17), δ 48.493 (C-18), δ 48.159 (C-19), δ 151.135 (C-20), δ 27.596 (C-21), δ 38.886 (C-22), δ 25.334 (C-23), δ 15.523 (C-24), δ 16.145 (C-25), δ 15.98 (C-26), δ 14.719 (C-27), δ 16.282 (C-28), δ 109.477 (C-29) and δ 18.490 (C-30) (Haque *et al.*, 2008).

MASS spectra of isolated compound from the methanol extract of stem of *Butea monosperma* (Lam.) Kuntze

The bioactive isolated compound from BMME had given the molecular ion peak [M+1] 425.379 in ESI mass spectrum which suggested molecular formula as **$C_{30}H_{50}O$.** (Haque *et al.*, 2008)

On the basis of phytochemical screening the isolated compound was found to be a Triterpene. The spectral data of FTIR, NMR and MASS were interpreted to predict the molecular structure, atomic stretching, possible molecular functional groups, etc. The compound thus interpreted was lupeol, a triterpenoid, isolated compound from stem of *B. monosperma*.



Structure of Isolated Compound TBM

Name of Isolated Compound

LUPEOL

IUPAC Name

(3β,13ξ)-Lup-20(29)-en-3-ol

Lupeol melting point 213°C which corresponds to the molecular formula $C_{30}H_{50}O$.

Figure 1: *Structure and IUPAC name of isolated compound TBM obtained from the methanol extract of the stem of Butea monosperma (Lam.) Kuntze*

8.2 Antidepressant Activity

Antidepressant activity of Isolated Compound TBM by Forced Swim Test (FST)

Table 2: *Antidepressant effect of the isolated compound TBM on FST induced depression And Duration of immobility (sec)*

Groups	**Duration of immobility (sec)**

	FST
Negative Control	131.70 ± 3.648
Fluoxetine 20 mg/kg	65.17 ± 4.362***
Isolated Compound 20 mg/kg	105.5 ± 7.343*

Values are expressed in Mean± SEM; ***p<0.001, *p<0.05, when compared to control group (one way ANOVA followed by Dunnett's 't' test), n=6, TBM= isolated compound obtained from the methanol extract of the stem of B. monosperma.

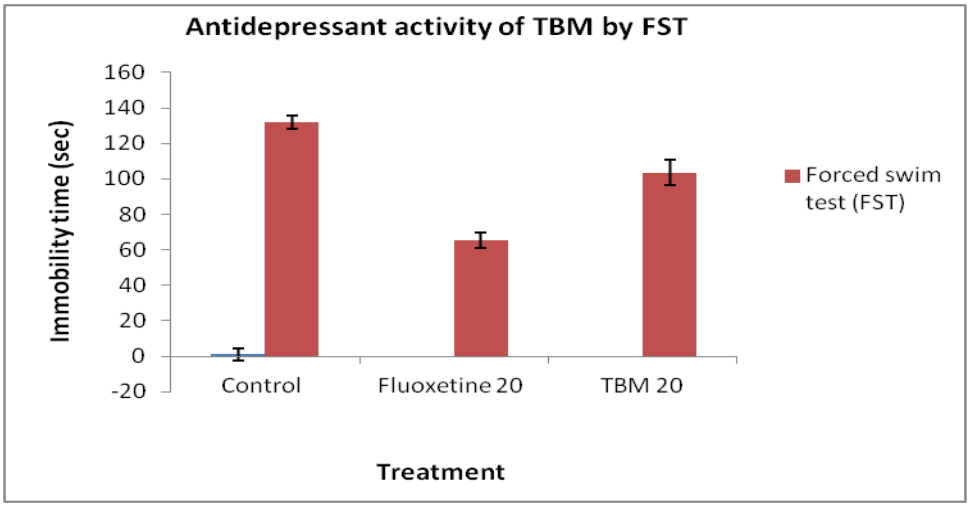


Figure 2: *Antidepressant activity of the isolated compound TBM on FST induced depression*

Antidepressant effect of the isolated compound had been studied to confirm the effect of TBM from BMME on depression induced mice. It was observed that TBM produced significant antidepressant effect (p<0.05), in the same fashion as that shown by BMME. These findings indicated the complex action of BMME on depression, as extract contained various active constituents which may have different pharmacological actions among them. The mechanism of TBM behind its antidepressant effect may be due to its effect on alteration of different biogenic amines.

8.3 Estimation of MAO-A in whole brain of Mice after treating with isolated compound TBM

Table 3: *Estimation of MAO-A in whole brain of mice*

Group	Treatment	MAO-A activity (U/g protein)
Normal Control	--	48.50 ± 2.527
Disease Control	Depression induced by FST	62.67 ± 3.159
Standard	Fluoxetine 20 mg/kg; bw	31.83 ± 1.973***
Isolated Compound	10 mg/kg; bw	44.17 ± 2.548***

Values are expressed in Mean± SEM; ***p<0.001, when compared to control group (one way ANOVA followed by Dunnett's 't' test), n=3. TBM= Isolated Compound obtained from the methanol extract of the stem of *B. monosperma.*

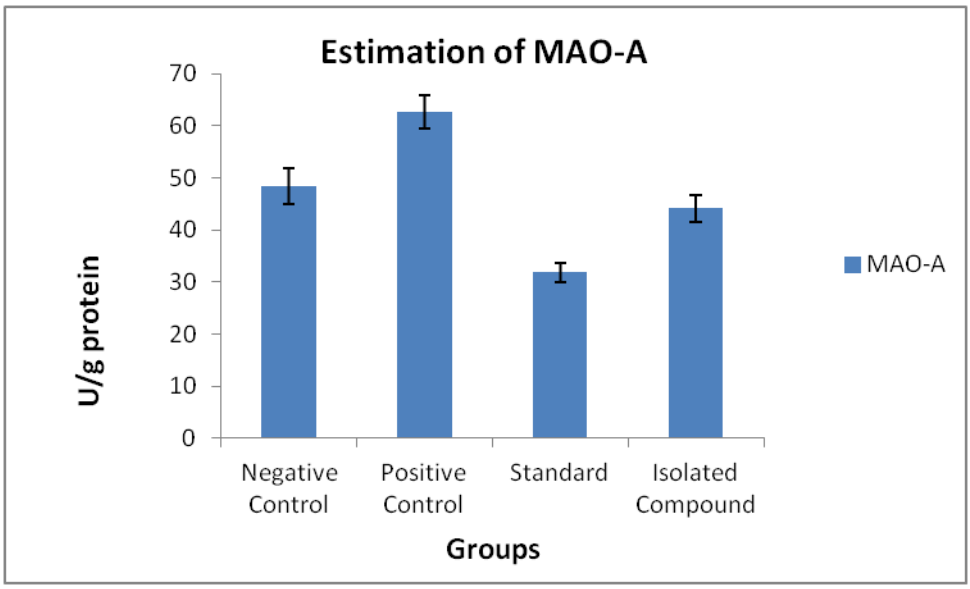


Figure 3: *Estimation of MAO-A in Mice brain by FST induced depression*

MAO-A is responsible for the degradation of biogenic amines in brain. It was already estimated that 5-HT level was decreased and NA level was increased in treated groups. MAO-A estimation (insignificant decrease in MAO-A level) confirmed the decreased metabolism of biogenic amines in mice brain. MAO-A levels were inhibited significantly in standard but

slightly decreased in isolated compound TBM treated animals. Increased level of NA and DA may be the main responsible factors behind the antidepressant effect of TBM from BMME.

9. Conclusion

In this present study it has been concluded that, the monoamines have a major role to control the depression in FST induced depressed mice.

It had been suggested by Reneric and Lucki that an increase in both swimming and climbing behaviors in the FST occurs when the animal is treated by a drug which increases NA and DA levels in the nerve terminals. An increase in NA and DA could be by inhibition of MAO activity in the brain.

Isolated bioactive compound from *Butea monosperma* (Lam.) Kuntze showed significant antidepressant activity may be due to the significant action on MAO.

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