



## CNS ACTIVITY OF HYDROALCOHALIC EXTRACT OF *BOMBAX CEIBA* FLOWER

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### ABSTRACT

*Bombax ceiba* Linn. (Malvaceae), commonly known as the cotton tree or red silk cotton tree, is a spectacular flowering tree with a height of up to 40 meters that is found in tropical and sub-tropical Asia as well as northern Australia. It has been chosen as the “city flower” of the cities of Kaohsiung and Guangzhou for its large, showy flowers with thick, waxy, red petals that densely clothe leafless branch tips in late winter and early spring. *B. ceiba* is a source of food, fodder, fiber, fuel, medicine, and many other valuable goods for natives of many Asian countries. For example, its fruits are good sources of silk-cotton for making mattresses, cushions, pillows. *Bombax ceiba* is a famous plant used extensively in traditional medicine for various diseases. However, data pertaining to its effects at CNS level is limited. To analyze the potential study of Hydroalcoholic extract *Bombax ceiba* flower was screened for locomotor, Rota-rod, Anticonvulsant, anti-anxiety activity of Hydroalcoholic extract (200 mg/kg and 400 mg/kg p.o.) was determined. The present study deals with various pharmacognostical examinations like organoleptic or macroscopical characters, microscopical or anatomical studies. Further studies are required to analyze the implicated phytochemicals and the mechanism at cellular level.

**Key words:** *Bombax ceiba*, locomotor, Rota-rod, Anticonvulsant, anti-anxiety activity.

### INTRODUCTION:

*Bombax ceiba* is a tree from the Malvaceae family which grows in Indonesia, Malaysia, China, Hong Kong and Taiwan. The tree has red blossoms with five petals. Tibetans refer to it as “salmari”, while in Europe and America, it is ordinarily referred as the cotton tree. It is utilized as a tea in parts of China. (Gupta R., 2013; Goyal, 2012). This tree is rich in various phytochemicals. Extracts have confirmed the

presence of alkaloids, flavonoids, glycosides, coumarins, proteins and amino acids. *Bombax ceiba* has numerous documented effects, some of which are anti-inflammatory, aphrodisiac, antimicrobial, hepatoprotective, anti-diabetic, anti-aging and hypotensive. Evaluation at the CNS level, however, is limited. (Maton et al., 1993).

Medicinal plant is the most basic and significant part of our medication system. Most of the medicines are made up of the herbal plant. It is also named as a medicinal plant. The herbal plant was used in our traditional medicine system for long ago. Countries in Asia and Africa 80% user used traditional medicinal system, which includes herbal or medicinal plant. (Vickers, 1999).

There are many uses and less or negligible side effects of traditional (medicinal plant) over any other system, for their healthy lifestyle. Many plants have medicinal properties. *Bombax ceiba* L. is also known as *Salmalia malabaricum*. generally, it is called as *Bombax ceiba*. It is having no. of common names. In Hindi, it is called as Semar, Semul, Semal, and in English, it is called as Red silk cotton tree, *Bombax ceiba* and in Marathi, it is commonly called as Kate savar, Semul. *Bombax ceiba* L. belongs to the family Bombacaceae Indian silk cotton (Gadge et al., 2012; Gandhare et al., 2010). It is naturally obtained in Pakistan, India, and Myanmar. But it is native or belongs to the western Africa. *Bombax ceiba* L. is a big and tall tree with a height of approximately 30 m and having a diameter of 58 to 78 cm. It grows in a straight direction with having a cylindrical stem and wide base.

The tree contains a very beautiful and large flower, which is pollinated mainly by birds because the flower contains nectar, which is used by birds. Moreover, studies on the cotton tree have shown that it produces many novel secondary metabolites and have explored its traditional medicinal usage by various tribal communities. The flowers are astringent and refrigerant. They are used to treat cutaneous troubles. The young roots are diuretic and

tonic. They are used in the treatment of cholera, tubercular fistula, coughs, urinary complaints, nocturnal pollution, abdominal pain due to dysentery, and impotency. The gum is astringent, demulcent and tonic. It is used in the treatment of dysentery, haemoptysis in pulmonary tuberculosis, influenza and menorrhagia. The leaves are hypotensive and hypoglycaemic (Chakraborty et al., 2010).

The ethnomedicinal activity of *bombax ceiba* L. Plant part Traditional medicinal uses, Thorn Used in the different formulation to treat Acne, Androecium Used for Food purpose, Petals for Skin and Cosmetics, Leaf for treatment of Diarrhoea, Larvicidal Activity, Root for the treatment of Piles, Bark Used for Wound Flower: The flowers show bitter action and also shows acrid cooling, dry, astringent to the bowels, anti-inflammatory action. (Shukla et al., 2020). It removes bile and phlegm of the body and purifies the blood; it is beneficial to the spleen and shows a good response in leucorrhoea. It is also used topically to skin affections as cooling and astringent. It is having phenolic compounds, Seeds show good action in chickenpox, smallpox, catarrhal affections, chronic cystitis, and genitourinary diseases. Bark Stem bark is used in the healing of wounds and as a paste in water to skin eruptions, boils, acne, pimples. The stem bark is also used as a demulcent, styptic, and used to removes phlegmatic. Aqueous extract with curd shows great action in treatment given for dysentery with blood. Leaves: It is used in the treatment of diarrhea and used in treatment for inflammation, larvicidal activity. Root bark is used for the treatment of piles. The root shows diuretic and astringent property. It is useful in biliousness, inflammations, and excessive heat of the body. Gum: The gum is an acrid, astringent, demulcent, tonic, aphrodisiac, and removes black bile. In powder form alone or with other herbs, it is used internally to treat

hemoptysis, diarrhea, dysentery, bleeding piles, menorrhagia, leucorrhoea, spermatorrhoea, and blood disorders. Topically it is applied as a styptic, astringent and demulcent in stomatitis, dermatological ailment, and burn wound. (Chaudhary and Khadabadi, 2012).

## MATERIAL AND METHODS

**Selection of plant:-** The plant selection on their availability and folk usage of the plant. The plant was chosen.

**Collection of Plant Material:** The Plant material of *bombax ceiba* was collected from Sehore Bhopal (M.P.), during the month of April 2021.

**Authentication of plant:** - The plant was identified And authenticated by Dr. Zia ul Hasan H.O.D. Department of Botany, Saifia Sciences College Bhopal (M.P.) and stored in the herbarium of the Institute and a specimen voucher no.312/Bot./Saf./21 was assigned.

**Defatting of plant material:** - The shade-dried plant materials are coarsely powdered and fats and oil removed by soxhlation process with petroleum ether. The extraction proceeded until the substance was defatted.

### Extraction by soxhlation process-

Accurately weight 135 gram of dried powdered flower of *Bombax ceiba* were extracted with Hydroalcoholic solvent using a 48- hour soxhlation procedure, filtered and dried with vaccum evaporator at 40<sup>0</sup>C, and prepared extract was also subjected to colour, odour and consistency.

**Determination of percentage yield of the extract:-** The crude extract after the soxhlation extraction process, extract was further on vaccum evaporater dried extract of flower of *Bombax ceiba* was done by using solvent Hydroalcoholic ( ethanol:water, 70:30 v/v). The percentage yield of extract were calculated 25 gm (18.51%).

## Quantitative phytochemical analysis

### Estimation of Total polyphenol content (TPC)

The total polyphenol content of the extract was estimated using the Folin Ciocalteau reagent based assay. 5-50 µg/ml methanolic gallic acid solutions were used as standards and methanol was used as a blank. The absorbance of the developed colour was recorded at 765 nm using a UV-Vis spectrophotometer. All determinations, for gallic acid as well as the plant extract, were carried out in triplicate. Data are represented as an average of the three determinations. Using these readings, a calibrated gallic acid standard curve was made. Based on the measured absorbance of the plant extract, the concentration of phenolics was estimated (µg/ml) from the calibration line. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material) (Bhalodia et al., 2011; Patel et al., 2012).

### Estimation of Total flavonoids content (TFC)

Total flavonoid content was based on aluminium chloride method. The 10 mg quercetin was dissolved in 10 ml methanol and various aliquots of 5,10,15,20 and 25 µg/ml

were prepared in methanol. And the 10 mg of dried extract of were dissolved in 10 ml methanol and filter. 3 ml (1 mg/ml) of this solution was used for the evaluation of flavonoid. In addition, 1 ml of 2 %AlCl<sub>3</sub> methanolic solution was added to 3 ml of extract or normal and allowed to stand at room temp. for 15 min. absorption was measured at 420 nm (Satish Kumar et al., 2008).

## PHARMACOLOGICAL ACTIVITY

Literature reveals that *Bombax ceiba* has been explored for its pharmacological activity

### Animals

Swiss albino mice weighing between 25-35 gm are used in the experiments. The animals were placed randomly and allocated treatment group. All the experiments were performed between 9:30 to 16:30 hours to overcome diurnal and circadian variations. All the animals were housed at a temperature of 25±2<sup>0</sup>C and in a relative humidity of 65±5%. A 12:12 light: day cycle was followed. All the animals were housed in polypropylene cages with paddy husk as bedding with free access to water and fed with standard commercial pelleted chow (Hindustan Lever). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee of Radharaman Institute of Pharmaceutical Sciences, Bhopal (M.P.) proposal number IAEC/Rips/2021/04 and were in accordance with the guidelines of the IAEC.

### Acute oral toxicity study.

The acute oral toxicity study was conducted according to the OECD-423 (Acute toxic class method) guidelines. Six group of mice n=3 were administered orally for 7 days with HEBC (50, 300,1000 and 2000 mg/kg, p.o.) and the animals were kept under observation for mortality and any behavioural changes. (OECD 2001)

### Effect of HEBC on locomotor activity of mice on Actophotometer.

Swiss albino mice weight 25-30 gm were taken and divided in groups each consisting of 6 animals. The first group was marked as control and second as standard group. Rest two groups were marked for different doses (200 and 400 mg/kg.p.o.) HEBC. The was turned on checked to make sure that all the photocell are working for accurate recording and each mice was placed individually in the activity cage for 5 minute. Basal activity score of all the animals were noted. Diazepam (2 mg/kg) was injected. and after 30 minute placed each mouse in activity case for 5 minute. Note the score, the difference in the activity before and after diazepam treatment. Repeat the above procedure for different doses of HEBC (200,400 mg/kg p.o.). Percentage change in motor activity was calculated. (Kulkarni, 2005)

### Effect of HEBC on muscle grip performance of mice on Rota-rod apparatus.

Swiss albino mice of about 30-35gm weight were taken and divided into 4 groups each consisting 6 animals. The first group was marked as control and second as standard group. Rest 2 group were marked for different doses (200 and 400 mg/kg. p.o.) of the HEBC.

Rota-road was turned on setting the speed of rotation at 22-25 rpm. The animals were placed singly one by one on rotating rod. The fall off time, when the mouse falls from the rotating rod was noted down.

The drug (diazepam, 2 mg/kg, i.p.) was injected to animal of second group and after 30 minute, the above mentioned parameter was observed. after that the same procedure was followed for the test group. Comparison was made between the fall-off time of all the animal. (Kulkarni, 2005)

#### **Effect of HEBC on parameter of anxiety on elevated plus- maze in mice.**

Swiss albino mice of about 25-35gm were taken and divided into 4 groups each consisting of 6 animals. The first group was marked as control and second as standard. Rest of 2 groups were marked for different doses of (200 and 400 mg/kg. p.o.) HEBC. Animals were placed individually at the centre of the plus maze with their head facing towards the open arm and their following behaviours were noted for five minutes. First preference of mice to open or enclosed arm. Number of entries in open and enclosed arms (An arm entry defined as the entry of four paws into the arm). Average time of each animal spends in each arm (Average time = total duration in the arm/number of entries). Standard drug (Diazepam 2 mg/kg. i.p.) and different doses of HEBC (200 and 400 mg/kg. p.o.) was injected to the animals of 3<sup>rd</sup> and 4<sup>th</sup> group and after 30 minutes. The above mentioned parameters were observed. Comparison were made among the preferences of the animal to open/enclosed arm. average time spent in open arm and

number of entries in open arm for each group (Kulkarni, 2009).

#### **Effect of HEBC on MES induced convulsion in rat.**

Swiss albino mice of about 25-35 gm were taken for experiments. Animals were marked and divided in 5 groups. each group consisting of 6 animals. First group was marked control and second and third group were designated for standard drug treatment (Phenytoin 120 mg/kg i.p.). Rest 2 group were marked for 2 different dose of HEBC (200 and 400 mg/kg. p.o.) respectively. Care was taken to hold the animal properly. Corneal electrodes were placed on the cornea and a current of 150 mA was applied for a duration of 0.2 sec. Different stages of convulsions i.e. (a) tonic flexion. (b) tonic extensor phase. (c) clonic convulsions. (d) stupor, and (e) recovery or death was noted after electric current application. The time (sec) spent by the animal in each phase of the convulsions was noted. The same procedure is repeated with all animals of the group. The standard drug and HEBC injected to the animals of all respective groups. After 30 minutes the same current was applied for similar duration and time spent in different stages was noted. The reduction in time or abolition of tonic extensor phase of MES-convulsions forever groups was noted (Kulkarni. 2005).

#### **RESULTS AND DISCUSSION:**

The hydroalcoholic extract of *Bombax ceiba* show the presence of steroid, tannins and phenolic compounds., alkaloids, glycoside, carbohydrate etc. The results are shown in table-1 The Percentage yield of hydroalcoholic extract was 25 gm (18.51% ). This study was conducted on several central nervous systems

related experimental models e.g., locomotor activity, rotarod, elevated plus maze, and MES induced convulsion to investigate the possible central effect of *Bombax ceiba*. The classical models for screening CNS action providing information on depressant property of psychomotor performance, anxiolytic and myorelaxant activity. There has been a considerable popular interest in the use of the natural remedies or herbal products to treat anxiety and depression. Recently several plants have been reported to possess anxiolytic effects in different animal models of anxiety. Various traditional herbal medicines have also been suggested to possess anxiolytic activity. The plant was found to be rich in steroidal and flavonoid content. The phytoconstituents which are responsible for many pharmacological activities.

Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity. *Bombax ceiba* significantly decreased locomotor activity in all the tested doses that act as a centrally acting muscle relaxant interacting with specific receptors enhancing chemical and mission. Decrease in locomotion reveals a depressant effect on GABAergic transmission due to an increase in the concentration of GABA in the brain (Sharma et al. 2012).

The HEBC CNS depressant reduces grip strength and mice may fall from the rotarod due to loss of muscle or muscle coordination. HEBC decreases the fall-off time of mice from the rotating rod. Based on the exposure of the animal to an elevated plus maze, the fear due to height induces anxiety in the animals when placed on the elevated plus maze (EPM). The

animal being exposed to the new environment tends to avoid and prefers to stay in the closed arm due to fear (Vishwanatha et al., 2009).

The ultimate manifestation of anxiety and fear in the animals is inhibited by a decrease in the motor activity and preference to remain in safer places. Anxiolytic spent by the animal in the open arms (Sharma et al., 2012). An anxiolytic effect expressed by an increased number of open arm entries and time spent in the EPM. Diazepam produced a significant increase in open arm duration and also number of entries into the open arms. The plus maze model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances acting at the GABA-benzodiazepine complex (Chakraborty et al., 2010). Current study data are consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce significant anxiolytic effects in a variety of anxiolytic screening procedures, including the elevated plus-maze test procedures. In our finding, the HEBC treated

Epilepsy is one of the most common serious neurological conditions. Drugs that inhibit voltage-dependent Na<sup>+</sup> channels, such as phenytoin. The effect of HEBC on MES-induced convulsion in rats is tabulated in table -6. The tonic and extension phase was decreased in a dose-dependent manner. The treated group showed a change in duration of clonus phase in all the HEBC treated groups, which was non-significant compared to the control. The animals were recovered in the vehicle-treated, phenytoin, and all doses of HEBC.

**Table 1: Qualitative analysis of *Bombax ceiba* hydroalcoholic extract of presence of different phytoconstituents.**

S.NO.	TEST	OBSERVATION	INFERENCE
1	<b>Alkaloid</b>		
	Wagner's reagent	Reddish brown ppt	+ ve
	Dragendorff's reagent	Reddish brown ppt	+ ve
	Mayer's reagent	Cream colour ppt	+ ve
2	<b>Glycoside</b>		
	Keller Killiani test.	Appearance of reddish brown colored ring at the junction of two layers	+ ve
	Conc. sulphuric acid test	reddish color precipitate	+ ve
	Molish's test	Formation of reddish-purple colored ring at the junction of two layers.	+ ve
3	<b>Steroid</b>		
	Solkowski Test	brown or red colored ring on the sulphuric acid layer given the confirmatory test.	+ ve
	Libermann Burchard's Test	translucent green colour given the confirmatory test.	+ ve
4	<b>Carbohydrates</b>		
	Molisch Test	Formation of the red violet ring at the junction of the solution and its disappearance on addition of excess alkali solution indicates the presence of carbohydrates.	+ ve
	Benedict's Test	Depending on the concentration of the reducing sugar, the amount and colour of the precipitate produced varied. A positive Benedict's test appears green, yellow, orange, or red.	+ ve

5	<b>Phenolic compounds</b>		
	Ferric chloride test	Formation of blue, green or violet colour indicates the presence of phenolic compounds.	+ ve
	Lead acetate test	Formation of white precipitate indicates presence of phenolic	+ ve
	Dilute iodine solution test	Formation of transient red colour indicates the presence of phenolic compounds	+ ve

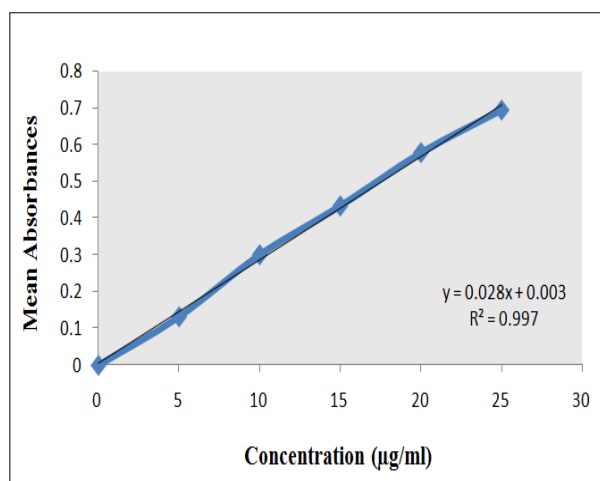


Figure 1: Calibration curve of Gallic acid

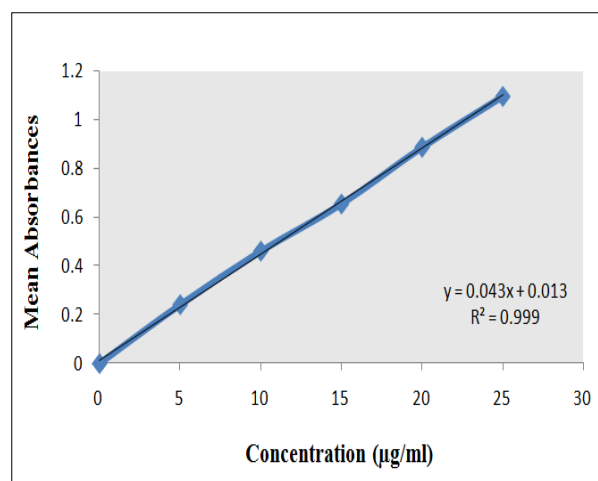


Figure 2: Calibration curve of Quercetin

Table 2: Estimation of total phenols and flavonoid content

Extract	Total phenols content (GAE mg/100mg)	Total flavonoid content (QE mg/100mg)
Hydro alcoholic extract of <i>Bombax ceiba</i>	0.215	0.335



**Table:-3 Effect of HEBC on locomotor activity of mice on Actophotometer**

Groups	Dose (mg/kg)	Locomotion Score (M±SEM) (Min.)		% Change in locomotor activity
		Basal	After 30 min. drug administration	
Vehicle control	5 ml/kg/p.o.	1132.5±137.50	-	-
Diazepam	2 mg/kg/i.p.	156.5±8.50	79.5±10.50**	92.98
HEBC	200 mg/kg/p.o.	162.5±2.50	252.5±97.50 <sup>ns</sup>	77.74
HEBC	400 mg/kg/p.o.	29±11.00	173±27.00 <sup>ns</sup>	84.72

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at \*\*\*P<0.001, \*\*P<0.01, \* P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey's post hoc test).

**Table:- 4 Effect of HEBC on muscle grip performance of mice on Rota-rod apparatus**

Groups	Dose (mg/kg,)	Fall off time in Sec. (M±SEM)		% Change in fall off time
		Basal reaction time (M±SEM)	After 30 min. drug administration (M±SEM)	
Vehicle control	5 ml/kg/p.o.	1066±41.00	-	-
Diazepam	2 mg/kg/i.p.	500±5.40	70±16.00***	93.43
HEBC	200 mg/kg/p.o.	1155.5±142.50	357±10.50**	66.51
HEBC	400 mg/kg/p.o.	175±10.00	192.5±12.50***	81.98

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at \*\*\*P<0.001, \*\*P<0.01, \* P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey's post hoc test).

**Table:-5 Effect of HEBC on parameter of anxiety on elevated plus- maze in mice.**

Groups	Dose (mg/kg,)	% preference to open arm	Total No. of entries (M±SEM)	% open arm entries
Vehicle control	5 ml/kg/p.o.	41.01	27.17±2.14	-
Diazepam	2 mg/kg/i.p.	65.24	11.60±2.28**	57.30
HEBC	200 mg/kg/p.o.	42.13	18.42±1.93 <sup>ns</sup>	32.20
HEBC	400 mg/kg/p.o.	51.23	14.86±1.79*	45.30

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at \*\*\*P<0.001, \*\*P<0.01, \* P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey's post hoc test).

**Table:-6 Effect of HEBC on MES induced convulsion on rat**

Group	Dose mg/kg	Flexon phase in sec. (M±SEM)	Extensor phase in sec. (M±SEM)	Clonus phase in sec. (M±SEM)	Stuper phase in sec. (M±SEM)	Recovery/ Death
Vehicle control	5 ml/kg/p.o.	11.5±1.50 <sup>ns</sup>	13.5±1.50 <sup>ns</sup>	23.5±1.50 <sup>ns</sup>	350±1.30 <sup>ns</sup>	Recovery
Phenytoin	120mg/kg/i.p.	Absent	Absent	13.5±6.50 <sup>**</sup>	172±0.50 <sup>*</sup>	Recovery
HEBC	200 mg/kg/p.o.	12.5±0.50 <sup>ns</sup>	5.5±0.50 <sup>ns</sup>	27±2.00 <sup>ns</sup>	190±30.01 <sup>ns</sup>	Recovery
HEBC	400 mg/kg/p.o.	14±1.00 <sup>ns</sup>	2.5±0.50 <sup>**</sup>	20.5±5.50 <sup>*</sup>	213.5±0.50 <sup>**</sup>	Recovery

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at \*\*\*P<0.001, \*\* P<0.01, \* P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey's post hoc

## CONCLUSION

Pharmacological investigation of the plant *Bombax ceiba* produce depressant action on the CNS. hydroalcoholic extract of *Bombax ceiba* induce act as hypnotic, also decrease anxiety, means act as anxiolytic agent due to hypnosis. *Bombax ceiba* also exert muscle relaxant and locomotor, anti-anxiety effect of mice. It is pharmacological safe with good bioavailability with least toxicity *Bombax ceiba*. This review gives some phytochemicals as well as the detailed pharmacological information of *Bombax ceiba*. The main focus on the pharmacological potentials of *Bombax ceiba*, which is very helpful to researcher to add more about this valuable plant. Apart from this still there are few options to investigate the unexplored potential of plant based on its uses. The active constituent needs to be isolate and should be considered for further in-vivo or in-vitro studies to confirm the traditional claims and to explore the potential of development of drug.

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