

EVALUATION OF BEHAVIORAL ACTIVITY OF *BACOPA MONNIERA* AND *SYZYGIUM CUMINI* IN COMBINATIONDalal Pramod Homdeo^{1*}, Duragkar Nandakishor Jiwanrao¹, Bodele Sishupal Bhanudas¹,
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ABSTRACT

Stress is a major factor responsible for behavioral disorder. The main objective of the present work is to investigate the evaluation of behavioral activity of *Bacopa monniera* (aerial part) and *Syzygium cumini* (seed coat), individually and in combination by using different extracts (viz; ethyl acetate, acetone, methanol and methanol: water). Three different models which are used for behavioral activity such as Forced swim test (FST), Elevated plus maze method (EPM), Open field test (OFT). The result of FST indicated that immobility time significantly decreased in group treated with combination of methanolic extract of *Bacopa monniera* and ethyl acetate extract of *Syzygium cumini* in dose (200/40 mg/kg) as compared to control. The result of EPM indicated that percent no of open arm entries and time spent in group treated with combination of methanolic extract of *Bacopa monniera* and ethyl acetate extract of *Syzygium cumini* in dose (200/40 mg/kg) as compared to control. OFT indicates that number of occurrences at center and duration at center significantly increased in group treated with combination of methanolic extract of *Bacopa monniera* and ethyl acetate extract of *Syzygium cumini* in dose (200/40 mg/kg) as compared to control group. Therefore it is clearly proved that *Bacopa monniera* and *Syzygium cumini* plant in combination showed promising behavioral activity.

Keyword: *Bacopa monniera*, *Syzygium cumini*, behavioral activity**INTRODUCTION**

Herbals are being used, in medicine from time immemorial because they have fitted the immediate personnel need, they are accessible and inexpensive¹. Herbal medicines had proven to be great use in prevention of chronic disease. Natural products have traditionally provided most of the drug in use. Despite the achievements of synthetic chemistry and the advances towards rational drug design, natural products continue to be essential in providing medicinal compounds to great use². A pathological state of mind producing clinically physiological symptoms (illness) together with impairment in one or more areas of functioning of body³. Behavioral disorders are the major disorders seen in today's populations due to rapid process of urbanization. Stress is a major factor responsible for behavioral disorders in today's world. Stress can be described as the sum total of all reactions of the body, which disturb the normal physiological equilibrium and result in state of threatened homeostasis⁴. In the past few years, much interest has risen in the use of herbs in the treatment of both depression and anxiety. Researchers have aroused interest in many folk and herbal remedies to find new potential antidepressant with no troubling side effects. *Bacopa monniera* (Brahmi) is a small herb with purple flowers. It grows in wet and sandy areas and near streams in tropical regions. It is a creeping herb with numerous branches and small fleshy, oblong leaves. Flowers and fruits appear in summer. The stem and the leaves of the plant are used. *Bacopa monniera* has been reported to have several neuropsychopharmacological activities such as anxiolytic activity but anxiety occurs along with depression and is treated with an anxiolytic, antidepressant or both⁵⁻⁷. There have been several reports of drug possessing both anxiolytic and antidepressant activities this led us to investigate *Bacopa monniera* for behavioral activity.

Second plant *Syzygium cumini* (Jamun) is used in traditional system of medicine in India. It is an evergreen tropical tree, 60-100 ft. tall. Leaves leathery oblong-ovate to elliptic or obovate and 6-12cm long, the tip being broad and shortly pointed. The flowers are numerous, scented, pink or nearly white without stalks. Fruit oval to elliptic, 5-3.5cm long, dark or nearly black. It contains a single large seed. According to ayurveda, its bark is astringent to the bowels,

anthelmintic, bronchitis and asthma etc. The fruit of the plant had been used as astringent, spleen diseases, chronic diarrhoea, dysentery and tonic to the brain. It is extensively used for diabetes⁸⁻¹⁰. In present work, the main objective is to evaluate behavioral activity of their two plants in combination.

MATERIALS AND METHODS**Collection and authentication of plant material**

The plant of *Bacopa monniera* was collected from the local region of Nagpur district. The above material was botanically identified and confirmed from the Department of Botany, Nagpur University, Nagpur. The plant specimen was dried and its herbarium sheet was prepared and it is available in Department of Botany, Nagpur University, Nagpur.

Successive solvent extraction of plant material with various solvents:

The aerial part of *Bacopa monniera* and seed coat of *Syzygium cumini* were air dried in shade, under normal environmental conditions and then subjected to size reduction to get coarse powder. Such powdered material was charged into the Soxhlet apparatus, and extraction was carried successively with the solvents such as Petroleum ether (60⁰-80⁰), Ethyl acetate, Acetone, Methanol, Methanol + water (50-50). In case of extraction with hydro alcohol maceration for 7 days was carried in cold conditions. Each time before extracting with the next solvent, the powdered material was air dried below 50⁰C and then each extract was concentrated by distilling off the solvent to obtain the crude extract (residue). The drug was extracted with each solvent till complete extraction was effected. The above extracts of the plant material were subjected to preliminary phytochemical screening for the detection of various plant constituents¹¹.

Pharmacological screening**Animals**

Albino rats of either sex weighing between 200-250 g procured from MAHAVEERA Enterprises, Hyderabad, were used for the present investigation. Animal Ethical Committee approved experimental protocol under guidelines of CPCSEA, New Delhi. The rats were housed at controlled temperature (25±20C) with food, water *ad libitum*. From the phytochemical screening results, acetone and

methanol extracts of Brahmi and ethyl acetate, acetone and methanol extracts of Jamun were used.

Behavioral activity

In this set of experiment Behavior of rats were assessed using various animal Behavioral model like forced swim test (FST), elevated plus maze (EPM) and open field apparatus (OFT). Effect of different extracts of *Bacopa monniera* and *Syzygium cumini* on behavior of rats was studied.

Forced swim test (FST)

For this test animals were divided into seven groups, each containing 6 rats (initial screening)¹¹⁻¹²

Group I : Control(vehicle)

Group II: Standard (imipramine 15mg/kg i.p)

Group III: Acetone ext Brahmi (30mg/kg & 40mg/kg p.o. × 5 days)

Group IV: Methanol ext Brahmi (30mg/kg & 40mg/kg p.o. × 5 days)

Group V: Ethyl acetate ext Jamun (150mg/kg & 200mg/kg p.o. × 5 days)

Group VI: Acetone ext Jamun (150mg/kg & 200mg/kg p.o. × 5 days)

Group VII: Methanol ext Jamun (150mg/kg & 200mg/kg p.o. × 5 days)

For effect of different combination of Jamun and Brahmi on FST

Test animals were divided into five groups, each containing six rats

Control Vehicle treated

Standard (Imipramine 15mg/kg i.p)

Group I Ethyl acetate ext Jamun (200 mg/kg p.o. ×5 days)

Group II Methanol ext Brahmi (30mg/kg & 40 mg/kg p.o. × 5 days)

Group III Ethyl acetate ext Jamun/ methanol ext Brahmi (150mg/kg & 40 mg/kg p.o. ×5 days)

Group IV Ethyl acetate ext Jamun/methanol ext Brahmi (200mg/kg & 40 mg/kg p.o.×5 days)

Group V Ethyl acetate ext Jamun/ methanol ext Brahmi (250 mg/kg and 40 mg/kg p.o. ×5 days)

The forced swim test (FST) was developed by Porosolt and colleagues for rats and subsequently the mouse. The FST is a

behavioral test used to evaluate the potential efficacy of prospective antidepressant drug in rats. The test is based on the observation that rats, following initial escape-oriented movements, develop an immobile posture when placed in an inescapable cylinder of water. The rats was placed in a cylinder(45×20 cm) containing 38 cm water (25±20C), so that the rat could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Two swim session were conducted, an initial 15 min. pretest, followed by 5 min. test 24 h later. Drugs were administered after the pretest. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min. test period was noted.

Elevated plus-maze (EPM)

Test animals were divided into 5 groups, each containing 6 rats¹³

Control Vehicle treated

Standard (Diazepam 2mg/kg i.p)

Group I Ethyl acetate ext Jamun (200 mg/kg p.o. ×5 days)

Group II Methanol ext Brahmi (40 mg/kg p.o. × 5 days)

Group III Ethyl acetate ext Jamun/ methanol ext Brahmi (150 mg/kg & 40 mg/kg p.o. ×5 days)

Group IV Ethyl acetate ext Jamun/ methanol ext Brahmi (200 mg/kg & 40 mg/kg p.o. × 5 days)

Group V Ethyl acetate ext Jamun/ methanol ext Brahmi (250 mg/kg and 40 mg/kg p.o. ×5days)

Elevated plus-maze: The elevated plus maze consists of two open arms (15 × 5 cm) and two closed arms (15 × 5 × 12 cm). The open pairs are perpendicular to closed one, after 30 min of the administration of the drug, the animals were placed at the centre of the plus maze. The number of entries in either arm and time in seconds, spent by the animals in open arm were noted. The animals were fasted 18 hrs prior to the experiment. Extracts of different combination of *Syzygium cumini* and *Bacopa monniera* were administered orally 30 min before their placement on elevated plus maze apparatus. Each rat of different treatment group was placed individually at center of elevated plus maze apparatus with its head facing an open arm

Number of entries in open arm

$$\text{Percentage of open arm entries} = \frac{\text{Number of entries in open arm}}{\text{Total arm entries}} \times 100$$

Open field test

Test animals were divided into 5 groups, each containing 6 rats¹⁴⁻¹⁵

Control Vehicle treated

Standard (Diazepam 2mg/kg i.p)

Group I Ethyl acetate ext Jamun (200 mg/kg p.o. ×5 days)

Group II Methanol ext Brahmi (40 mg/kg p.o. × 5 days)

Group III Ethyl acetate ext Jamun/ methanol ext Brahmi (150 mg/kg & 40 mg/kg p.o. ×5 days)

Group IV Ethyl acetate ext Jamun/ methanol ext Brahmi (200 mg/kg & 40 mg/kg p.o. × 5 days)

Group V Ethyl acetate ext Jamun/ methanol ext Brahmi (250 mg/kg and 40 mg/kg p.o. ×5 days)

Open field test

The open field (50 cm diameter, 40 cm height walls). The floor has three concentric circles divided into 20 segments of equal area by lines radiating from the center. After 30 mins of the administration of the drug, the animals were placed individually in the center of the open field. The number of occurrence at center and perimeter were noted along with duration at center and perimeter.

RESULT AND DISCUSSION

Table 1: Effect of different extract of *Bacopa monniera* and *Syzygium cumini* extract on Forced swim test

Groups	Dose (mg/kg)	Immobility time (sec)
Control	-----	142±1.23
Standard	15 mg/kg(i.p)	46.8±1.8
Ethyl acetate extract (Jamun)	150 mg/kg	72.8±2.3
Acetone extract (Jamun)	150 mg/kg	93.6±1.8
Methanol extract(Jamun)	150 mg/kg	88.8±1
Acetone extract(Brahmi)	30 mg/kg	78.4±1.02
Methanol extract(Brahmi)	30 mg/kg	63.6±1.08
Ethyl acetate extract (Jamun)	200 mg/kg	65.3±2.1
Acetone extract (Jamun)	200 mg/kg	84.3±2.5
Methanol extract (Jamun)	200 mg/kg	70.5±2.2
Acetone extract(Brahmi)	40 mg/kg	72.6±1.9
Methanol extract(Brahmi)	40 mg/kg	68.7±2.8

Values are Mean ± S.E.M.

Table No.2: Effect of different combination of extract of *Bacopa monniera* and *Syzygium cumini* on forced swim test

Groups	Dose (mg/kg)	Immobility time (sec)
Control	-----	139±1.817
Standard	15mg/kg (i.p.)	46.8±1.8
Group I	200 mg/kg	70.3±2.1
Group II	40 mg/kg	68.7±2.8
Group III	150/40 mg/kg	78.3±0.89
Group IV	200/40 mg/kg	58.00±1.095
Group V	200/40 mg/kg	62.2±2.37

Values are Mean ± S.E.M.

Immobility time

The immobility time was found to be 139.2±1.817 sec in control group, the immobility time decreased to 70.3±2.1 sec in ethyl acetate extract (*Syzygium cumini*) and 68.7 ± 2.8 in methanol extract (*Bacopa monniera*) treated groups. immobility time in group treated

with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of 150/40mg/kg was found to 78.3±1.5 sec The immobility time was found to be significantly decreased to 58.00±1.095 sec in group treated with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of 200 mg/kg and 40 mg/kg respectively. The immobility time in group treated with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of 250mg/kg and 40mg/kg respectively, was found to be 62.2±2.37 sec. In standard group treated with imipramine (15mg/kg) the immobility time was found to be 46.18 ± 2.6 sec. The above result of FST indicated that immobility time, significantly decreased in group treated with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of 200 mg/kg and 40mg/kg respectively as compared to control group.

Table 3 : Effect of different combination of extracts of *Bacopa monniera* and *Syzygium cumini* on Elevated plus-maze

Treatment	Percentage of entries in open arm	Percentage of entries in closed arm	Time spent in open arms(sec)
Control	18.38 ± 2.20	81.60 ± 2.19	28.83 ± 0.6
Standard	38.93 ± 2.4	60.71 ± 2.4	67.67 ± 0.4
Group I	22.42 ± 1.38	78.44 ± 2.1	45.48 ± 1.9
Group II	26.42 ± 1.0	72.5 ± 1.09	48.22 ± 2.6
Group III	23.33 ± 1.38	76.67 ± 1.73	52.83 ± 0.47
Group IV	31.47 ± 1.73	68.53 ± 1.27	57.83 ± 0.7
Group V	30.00 ± 1.57	71.00 ± 1.57	57.50 ± 0.6

Values are mean ± S. E. M.

Percent number of open arm entries

The percent numbers of open arm entries in were found to be 18.68 ± 2.20, 22.43 ± 1.38, 26.23 ± 1.0, 23.33 ± 1.08, 31.47 ± 1.73, 30.00 ± 1.57 and 38.93 ± 2.4 in control group, group I, group II, group III, group IV, group V and standard group treated with diazepam (2mg/kg) respectively. The above results indicated that percent number of open arm significantly increased group treated with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of 200mg/kg and 40mg/kg respectively as compared to control group.

Time spent in open arms

The time spent in open arms were found to be 28.83±0.6 and 67.67±0.4 in control group, group I, group II, group III, group IV, group V and standard group treated with diazepam(2 mg/kg)respectively. The above results indicate the time spent in open arms significantly increased in group treated with combination with *Syzygium cumini* and *Bacopa monniera* in the dose of 200mg/kg and 40mg/kg as compared to control group

Table 4 : Effect of different combination of extracts *Bacopa monniera* and *Syzygium cumini* on Open field test

Treatment	No of occurrences center	No of occurrences at perimeter	Duration at centre (sec)	Duration at perimeter (sec)
Control	6.67 ± 1.033	10.0±0.89	22.50±1.5	268.5±1
Standard	13.33 ± 0.81	6.33±0.81	47.33±2.06	242±3.0
Group I	7.02 ±1.33	9.18±2.1	27.42±1.5	261.1±1.9
Group II	8.9±1.08	8.22±1.24	28.1±1.44	258.2±1.2
Group III	7.500±1.04	9.17±0.9	26.15±1.04	265±2.1
Group IV	10.83±0.75	7.00±0.89	34.37±1.21	250±1
Group V	10.80±0.74	6.97±0.30	33.67±1.2	252±1

Values are mean ±S.E.M.

Number of occurrences at centre and perimeter

The no of occurrence at center were found to be 6.67 ± 1.033, 8.9 ± 1.08, 7.500 ± 1.04, 10.83 ± 0.75, 10.83 ± 0.74, 13.33 ± 0.81 in control group, group I, group II, group III, group IV, group V and standard group treated with diazepam (2mg/kg) respectively. The no of occurrences at Perimeter were found to be 10.0 ± 0.89, 9.18 ± 2.1, 8.22 ± 1.24, 9.17 ± 0.9, 7.00 ± 0.89, 6.97 ± 0.30 and 6.33 ± 0.81 in control group, group I, group II, group III, group IV, group V and

standard group treated with diazepam (2mg/kg) respectively. The above results indicated that no. of occurrences at centre significantly increased in group treated with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of (200/40mg/kg) as compared to control group and no. of occurrences at perimeter was significantly decreased in same group.

Duration at center (sec)

The duration at center (sec) was found to be 22.50 ± 1.5 , 27.40 ± 1.5 , 28.1 ± 1.44 , 26.50 ± 1.40 , 34.67 ± 1.21 , 33.67 ± 1.21 , and 47.33 ± 2.06 in control group, group I, group II, group III, group IV, group V and standard group treated with diazepam (2mg/kg) respectively. The above results indicated that duration at center (sec) significantly increased in group treated with combination of *Syzygium cumini* and *Bacopa monniera* in the dose of (200/40 mg/kg) as compared to control group.

CONCLUSION

In the present work, the attempt was made to study behavioral activity of *Bacopa monniera* and *Syzygium cumini* in combination. For this aerial part of *Bacopa monniera* and seed coat of *Syzygium cumini* were selected. The result proved that *Bacopa monniera* and *Syzygium cumini* plant in combination showed promising behavioral activity.

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