

ANTIDEPRESSANT AND ANTIOXIDANT ACTIVITY OF ETHANOLIC POLYHERBAL EXTRACT USING ANIMAL MODEL

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ABSTRACT

The present study was undertaken to evaluate the antioxidant and antidepressant activity of ethanolic Polyherbal extract. The ethanolic extracts were prepared by using leaves of *Bacopa monnieri* and fruits of *Terminalia chebula* by the maceration and percolation extraction process. Drugs were administered orally as per mg/kg body weight, one time for seven days. Antidepressant activity evaluated by force swim test and antioxidant activity by DPPH assay. The force swim test showed remarkable antidepressant activity at 250mg/kg in combination of BM and TC showing decrease in immobility time. Antioxidant activity by DPPH showing Radical Scavenging Activity of 94.40385% at 50µg/ml and IC₅₀ of 38.8614 µg/ml.

KEYWORDS: *Bacopa Monnieri* (BM), *Terminalia chebula* (TC), Depression, Antioxidant.

INTRODUCTION

During the last decade much attention has been focused on the role of reactive oxygen species in numerous diseases. Free radical technology causes cumulative damage to DNA, proteins, and lipids caused by oxidative stress. This oxidative pressure has been advised to purpose developing older and several human illnesses, which includes maximum cancers, hepatic problems and diabetes. DNA damage mediated with the aid of using manner of free radicals may also bring about mutation or chromosomal aberrations foremost to carcinogenesis.^[1] The utilization of the medicinal herb within side the treatment and prevention of illnesses is attracting interest through scientists globally. Active oxygen species and unfastened radicals play role within side the initiation and evolution of excessive sickness.^[2,3] In a normal cell there is an appropriate pro-oxidant, antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged.^[4]

The use of compounds with antioxidant activity is expected to be useful for these diseases. Therefore, there has been a developing interest in locating novel antioxidants to fulfill the requirements of pharmaceutical industries. Among the various healthful and preparation plants, some endemic species are of specific interest because of the actual fact they will be used for

generating raw substances or preparations containing phytochemicals with big antioxidant capacities and fitness benefits. Reactive Oxygen species had been discovered to play an crucial operate within the initiation or development of diverse diseases that embody atherosclerosis, inflammatory injury, most cancers and vessel disease

According to the World Health Report, about 450 million people suffer from mental or behavioral disorders. By 2020, depression will be the second largest cause of the global illness burden after heart disease. . Depression is a systemic condition that affects not only mood and emotions, but also the body and thinking process. Depression is a heterogeneous mood disorder that has been classified and treated in a variety of ways. Although a number of synthetic drugs are used as standard treatment for clinically depressed patients, they have side effects that can interfere with therapeutic management. Therefore, it pays to look out for herbal antidepressants with proven benefits and a favourable risk-benefit ratio. Several medicinal plants and medicines derived from these plants have demonstrated antidepressant properties due to the combined effects of their medicinal components. The causes of depression are decreased brain levels of monoamines such as norepinephrine, dopamine and serotonin .Therefore, drugs that restore reduced levels of these monoamines in the brain, either by inhibiting monoamine oxidase or by inhibiting the

reuptake of these neurotransmitters, could be fruitful in treating depression.^[5,6]

Bacopa monnieri L. (Fam. Scrophulariaceae) is a creeping, glabrous, succulent herb, rooting at nodes, allotted at some stage in India in all undeniable districts, ascending to an altitude of 1320 m. The plant is mentioned to expose sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity.^[7]

In Bangladesh, this plant is extensively utilized in the normal drugs system as potent therapeutic agent as a medical specialty tonic to reinforce intellectual development, to treat brain disease, internal organ, metabolism and biological process disorders, aching and purifies blood. In some components of this country Brahmi is employed to treat rheumatism and to forestall miscarriage. Some researchers isolated a number of these phytochemicals adore Brahmin, nicotine, herpestine, des-saponin glycosiditerpenoid saponins like Bacosides A & B. It's been found that the phytochemicals like 3-(α -L-arabinopyranosyl)-O- β -D-glucopyranoside-10 and 20-dihydroxy-16-keto-dammar-24-ene (Bacosides A & B) are the main compounds that will exhibit neuropharmacological activities by directly performing on the neurotransmitter level.^[8]

Terminalia chebula (Family: Combratacea) is a medium-huge sized tree observed in sunny forests of Asia. The plant is broadly called black- or chebulic myrobalan in English. In Sanskrit, it's miles referred to as as 'Haritak' that referees away from all of the diseases.² The fruit of the plant has massive medicinal values and may be observed within side the Ayurvedic, Chinese and Tibetan remedy literatures., It's miles a major constituent of an a conventional natural remedy triphala, that's doubtlessly powerful for numerous medical makes use of in Ayurvedic system. Several pharmacological sports were mentioned for *T.chebula*. Earlier preclinical research propose that management of the crude drug extract possesses antianaphylactic, antispasmodic, prokinetic, immunosuppressive, cardiotoxic, antioxidant, antihepatotoxic, antimicrobial, antimutagenic/anticarcinogenic, cytoprotective, radio protective, antidiabetic and retinoprotective activitiesAnxiolytic-like and antidepressant-like results.^[9]

MATERIALS AND METHODS

The Leaves of *Bacopa Monnieri* and fruits of *Terminalia Chebula* were collected from Botanical garden of Seth Govind Raghunath Sable College Of Pharmacy Saswad, Pune.

Extraction Procedure for *Bacopa monnieri* and *Terminalia Chebula*

Leaves were collected and shade dried and coarse ground powder was prepared Leaves of bramhi About 250 g of powdered material have to be soaked in 800 mL ethanol at 25 ± 2 °C for 72 h in a beaker and mixture needs to be stirred every 18 h using a sterile glass rod. Filtrate was

obtained 3 times with the help of Whatman No. 1 filter paper and sterilized cotton filter. The solvent was removed by rotary evaporator and 8.40 g extract.

The collected fruits were washed with running tap water to remove the adhering debris, after drying the fruits were grounded to coarse powder using electric grinder, then plant material is soaked in ethanol for overnight Ethanol is used as menstruum .Plant material (powder i.e. 500 g) is then percolated with circulating (95% of ethanol 200 ml) for three rounds and repeated for three days then residue extracted again twice using same procedure and then combine extract was filtrated and dried under reduce pressure and obtained yield is 44.56 grams.

Experimental animals

Animals Swiss Albino mice weighing 22-35 grams were housed in standard laboratory conditions (25 ± 5 °C; 55% humidity) and natural light/dark cycle with free access to standard pellet chow and drinking water ad libitum. All experimental procedures were carried out within the light period of the light/dark cycle. The experimental protocol was in strict accordance with regulations and prescribed animal ethical procedures outlined by the Institutional Research Committee Experimental protocol no. SGRS/IAEC/01/2020-21 was approved by the Institutional Animal Ethics Committee.^[10]

Effect of ethanolic Polyherbal extract of *bacopa monnieri* and *terminalia chebula* on depression:

The animals were ranged in five groups each containing 6 animals.

Group 1: (control group): treated with cmc.

Group 2: (standard): Imipramine 15mg/kg.

Group 3: ethanolic Polyherbal extract of *Bacopa monnieri* 60mg/kg+*Terminalia chebula* 40mg/kg

Group 4: ethanolic Polyherbal extract of *Bacopa monnieri* 100mg/kg+*Terminalia chebula*100mg/kg

Group 5: ethanolic Polyherbal extract of *Bacopa monnieri* 250mg/kg+*Terminalia chebula*250mg/kg

Administration of drugs

CMC (carboxy methyl cellulose) in distilled water was used as vehicle for dose administration. Drugs were administered orally as per mg/kg body weight, one time a day for 7days.^[11]

Drugs and chemicals

Drugs and chemicals Imipramine 15 mg/kg was used as a standard drug. Ethanol, distilled water, CMC, ethanol, methanol, DPPH was obtained from the laboratories of PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, Maharashtra.

Force Swim test

Swiss albino mice of either sex are used. They are brought to the laboratory at least one day before the experiment and are housed separate cages with free

access to food and water. Rats or mice rats are individually forced to swim inside a vertical Plex glass cylinder. After 5–6 min immobility reaches a plateau where they remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test.^[11]

Invitro antioxidant activity

DPPH radical- scavenging activity

The stable 1,1-diphenyl-2-picryl hydroxyl radical (DPPH) was used for determination of free radical-scavenging activity of the extract. The addition of 0.1 m DPPH solution in various concentrations (10, 20, 30, 40, and 50 µg/ml) of plant extract/ascorbic acid in the presence of Tris -HCl buffer (50 mM, pH 7.4), which was measured at 517 nm. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. & experiments were performed in triplicate. & DPPH radical scavenging activity was estimated using the equation. Mixture of methanol and extract served as the blank. The per cent inhibition was calculated by measuring the absorbance of

extract/ascorbic acid treated samples against the blank. The IC₅₀ values for the Polyherbal extract were calculated and compared with the standard reference compound ascorbic acid. The data for both extraction methods were collected and tabulated.^[12]

DPPH scavenging effect (%): % Inhibition= $\frac{A_0 - A_1}{A_0} \times 100$

Where A₀ = The absorbance of control.

A₁ = The absorbance of sample. As DPPH was soluble in methanol, it was taken up as organic phase.

Statistical Analysis: A one-way analysis of variance was used, followed by Dunnett's multiple tests, for statistical analysis. For each group of six rats, the results are expressed as the mean, standard deviation. Differences between groups were deemed statistically significant at the P 0.0001 level.

RESULTS AND DISCUSSION

Results: Effect of ethanolic Polyherbal extract of bacopa monnieri and terminalia chebula on depression by using Force swim test.

Table 1: Results of Force Swim Test.

Group	Treatment group of mice	Immobility period in (sec) Day 1	Immobility period in (sec) Day 7
1	CMC	226	230
2	Standard (Imipramine15mg/kg)	189±1.632	160±1.83****
3	TC40mg/kg+BM60mg/kg	209±1.471	188±1.33****
4	TC100mg/kg+BM100mg/kg	198±1.87	175±1.573****
5	TC250mg/kg+BM250mg/kg	159±1.472	144±1.978****

Each value represents the mean ± SEM (n = 6), Data was analysed using one-way ANOVA followed by Dunnett's multiple comparison test. **** p < 0.0001 compared with control. (where TC is Terminalia chebula, BM is Bacopa monnieri)

- Table 1 exhibited that ethanolic Polyherbal extract of doses of 250mg/kg shows significant decrease in immobility**** p < 0.0001 period as compared to day 1, Each dose of TC 40mg/kg and BM60mg/kg and TC100mg/kg and BM 100mg/kg show reduction in immobility when compared to cmc i.e. is control with increase in dose immobility reduced respectively.

- The force swim test was performed and immobility of mice measured with respective to time In normal group immobility period is of 226 sec and of standard is 175 sec. Similarly movement observed in different doses of combination of and at increasing doses should decrease in immobility time as compared with control. As we see that there is reduce in immobility time from day1 to day 7 as the dose TC 250mg/kg +BM 250mg/of shows remarkable effect reduction in immobility.

Results of DPPH assay

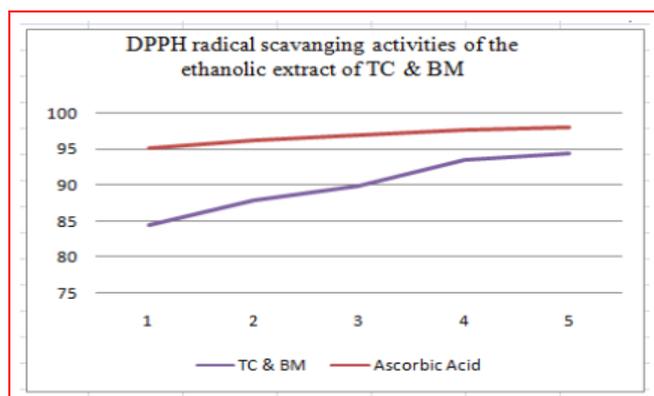
Absorbance of Terminalia chebula and Bacopa monnieri in combination in different concentrations:

Table 2: Absorbance of ethanolic Polyherbal extract at varying concentrations.

Concentration(µg/ml)	Absorbance
10	0.0812
20	0.0628
30	0.0532
40	0.0335
50	0.0291

Table 3: % Radical scavenging activity at various concentrations.

Concentration(µg/ml)	%RSA
10	84.38462
20	87.92308
30	89.76923
40	93.55769
50	94.40385



Graph 4: Comparison with ascorbic acid.

Table 4 Represents Graphical presentation compared with ascorbic acid.

Ethanolic extract of leaves of bacopa monnieri and fruits of terminalia chebula had remarkable scavenging effect on DPPH free radical which increase with increasing in concentration from 10 μ g/ml to 50 μ g/ml. The scavenging effect increase significantly as at 50 μ g/ml shows 94.40385% of radical scavenging activity at absorbance 0.0291 The IC₅₀ value of this Polyherbal extract is 38.8614 μ g/ml. The IC₅₀ value (inhibitory concentration of extract required to inhibit 50% of initial DPPH free radical) of plants ethanolic extract and ascorbic acid as positive control was determined graphically from graph of DPPH inhibition in table no 4.

Ethanolic Polyherbal extract of leaves of Bacopa monnieri and fruits of Terminalia chebula shows various pharmacological effect in various kinds of diseases. As Bacopa monnieri is considered to be nervine tonic and in ayurvedas it is called as Medhya Rasayana which affects on intellect and disorders associated with it and shown good activity in depression due to presence of Brahmin, nicotine, herpestine, des-saponin glycosiditerpenoid saponins like Bacosides A & B showing effect on neurotransmitter level may be by increase in effect of serotonin and norepinephrine^[13] and Terminalia Chebula known as King of Medicines and as part of Triphala churna and various uses and also its activity to get rid out of depression due to presence of Chebulinic acid showing antidepressant activity so the combination of these two medicinal herbd shows remarkable antidepressant and antioxidant activity.^[14]

The 250mg/kg dose of TC and BM showing reduction in immobility with increas in dose causing antidepressant activity. these both herbs produce amazing diversity of secondary metabolites , the Phytochemical investigations and invitro antioxidant activity of TC and BM indicated that as increase in concentration shows highest radical scavenging activity of 94.40385 at 50 μ g/ml and IC₅₀ of 38.8614 μ g/ml showing significant potency for antioxidant activity

CONCLUSION

From this research conducted, it may be concluded that the ethanolic Polyherbal extract of leaves of Bacopa monnieri and fruits of Terminalia chebula shows various pharmacological effect in depression and oxidation may be due to presence of alkaloid terpenoid, flavanoid. An approach was made to identify and quantify the amount of phytochemicals probably responsible for the antioxidant activity, and it was found rich in these phytochemicals and it can be a good source not only for the antioxidant but also for the treatment of depression. The present results are supportive and useful for their application as Positive drug candidate if further research in this direction with proper clinical trial support.

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