IN-VITRO FREE RADICAL SCAVENGING AND ANTICANCER POTENTIAL OF METHANOL LEAF EXTRACT OF PLANT BACOPA MONNIERI AGAINST HCT15- CELL LINE

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Abstract - It is a universal fact that all living organisms are depending on plants for breathe, food, medicinal and everyday necessities. So, since the period of time immemorial, the peoples are using plant as a medicine to cure various diseases without knowing the scientific back-ground beyond this. The plant Bacopa monnieri (L.) Wettst, comes under the family Scrophulariaceae are commonly known by different names such as Water hyssop, Baby’s tear, Brahmi, Jalbrahmi, Nira-brahmi and Saraswati. In Ayurvedic system of ancient civilization, this plant is used to treat anxiety, improving cognitive functions, memory enhancement, hepto-protection and neuro-protection. This practice is continuing even today in the village side. Hence, the present study has been designed to evaluate the phytochemical constituents, extractive value to determine appropriate solvent to get maximum phytocompounds and antioxidant potential of the plant Bacopa monnieri. The obtained results have seen the presence of various important secondary metabolites such as amino acids, steroids, glycosides, flavonoids, alkaloids and tannins in methanolic extract. The Cytotoxicity analysis by the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay method showed a significant decline in cell viability in the colon cancer cell line-HCT15 indicates the presence of anticancer activity for this plant extract. The anticancer potential of Bacopa monnieri increased with increasing concentrations of plant extract.

Key words: Bacopa monnieri (L.), free radical scavenging, anticancer, FTIR, GCMS, DPPH, FRAP, nitrous oxide, Phytocompounds

I. INTRODUCTION

India has a rich culture with good knowledge in usage of medicinal herbs and species. India is one of the 17 mega-biodiverse countries in the world. Nearly 50,000-70,000 medicinal plants were reported till date in India which leads to pave a way for very big exposure to traditional medicines such as Ayurvedic, Unani, Siddha from the ancient period. Even today majority of the people in rural India are using plant based traditional medicines to cure the various diseases. But, in this contest both the medicinal practitioners and patients are not aware about the chemical constituents of the plant and their disease curing potential. Till date a very few studies were conducted to identifying phytochemical and pharmacological potential to cure the diseases [1-2]. Recently the most of the researchers are involving in phytochemical study on plants to know their phytocompounds constituents, properties and various diseases [3]. Usually, in human being vast majority of diseases are caused by the free radical generation during metabolic processes. The natural defense of the human body against free radicals is not always sufficient mainly due to the significant exposure to pollutant substances. But, the medicinal plants having the rich antioxidants are very helpful to balance the accumulation of free radicals in human body. The plant Bacopa monnieri (L) listed the family Scrophulariaceae, has been used for more 3000 years as Indian Ayurvedic medicines for improving memory, increasing brain function, or promoting longevity [4-6]. Bacopa monnieri is a small creeping plant having numerous branches, fleshy, oblong leaves and small plant. Fruits and flowers are appearing in summer period the whole plant is medicinally important [7]. It is also possesses anti-inflammatory, analgesic,
antipyretic, epilepsy, insanity, anticancer and antioxidant activities [8-11]. In addition, the plant has been recommended as an agent for phytoremediation [12-15]. Hence, the present investigation has been programmed to evaluate the qualitative of phytochemical analysis, extractive value and antioxidant potential of Bacopa monnieri.

II. MATERIALS AND METHOD

A. Collection of plant material and authentication-
The whole plant of Bacopa monnieri (L) was collected from Nowlock garden, Avaraikkarai village, Ranipet area, Vellore District, Tamil Nadu, India. The plant sample was identified and authenticated by Institute of herbal science, Plant anatomy research Centre, Chennai, Tamil Nadu. The identification specimen number is (PARC\17\3535). The leaves were alone handpicked from plant and washed with distilled water to remove dust and other small insects stick on it. The cleaned leaves were dried for 30 days at room temperature (32°C) in shade place to avoid the loss of vitamins and denature of phytochemicals. After the completion of drying process, the shade dried leaves were powdered coarsely using electric blender. Then the powder was stored into air tight container for future uses.

B. Determination of Extractive value-
The dry Bacopa monnieri plant powder was extracted in different solvents such as water, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and hexane by using the maceration process. For this study, accurately 1gm of plant powder was taken in a small beaker (100ml) and 50ml of any one of above mentioned desired solvent was mixed with it. The entire setup was kept at room temperature for 24 hours. The mixture was shaken frequently. Thereafter, the extract was filtered using No.1 Whatmann filtered paper [16]. The filtrate was shifted to pre-weighed petri plates. Date and weight of the plate it were marked. The extract was kept upto complete evaporation of solvent. Finally, the dried extract containing petri plates was weighed. The extractive value (%) was calculated used by following formula.

Extractive value (%) = Weighed dried extract/ Weigh of plant material X 100

C. Preparation of the plant extract
Bacopa monnieri plant powder 20gm was packed in a thimble and 200 ml of methanol solvent was filled in the bottom of soxhlet. The heating mantle was set at 60°C [17]. After the completion of extraction, the methanolic leaf extract collected on the bottom were concentrated by evaporating it to dryness under reduced pressure by rotary vacuum evaporator to obtain the dried sample. The sample was stored at 180°C for further analysis.

D. Preliminary Qualitative phytochemical determination
The methanolic plant extract obtain from soxhlation process were subjected to preliminary qualitative determination for the presence of phytochemicals such as cardio glycosides, alkaloids, flavonoids, saponins, phenols, steroids, Proteins, Anthraquinones, coumarin, triterpenes, quinines and tannins by standard methods [18].

i. Test for tannins:
The methanol leaf extract of the plant Bacopa monnieri was taken in three separate test tubes. In each test tube few drops of 0.1 % ferric chloride were added. The brownish green or blue black colour was formed in test tube indicates the presence of tannin.

ii. Test for saponins:
1 ml of leaf extract and 2 ml of water was taken in a test tube. Along with few drops of Wagner’s reagent was slowly added. A prominent yellow colour precipitate was formed in test tube indicates the presence of saponins.

iii. Test for flavonoids:
1 ml of methanolic plant leaf extract was taken in test tube. Addition to this 1ml of NaOH was added. Now, the yellow colour formation in test tube formed into white colour after adding few drops of con. H2SO4 confirmed the presence of flavonoids.

iv. Test for alkaloids:
Drangandroff reagent
1 ml of Bacopa monnieri plant extract was taken along with, few drops of drangandroff reagent was added. A prominent yellow colour precipitates was formed in a test tube. It indicates presence of alkaloids as positive.

Mayer’s test
Take 1ml of plant extract in a test tube. Along with few drops of Mayer’s reagent were added. The white creamy was observed indicates the presence of alkaloid.

Wagner’s test
Methanolic leaf extract was taken in a test tube, along with few drops of Wagner’s reagent were slowly added. Creamish brown colour precipitate in a test tube confirmed the presence of alkaloids.

v. Test for protein:
1 ml of sample was taken. Along with few drops of Millon’s reagent was added. Appearing of white
precipitate in the test tube indicates the presence of Protein.

vi. Test for steroids:
1 ml of Bacopa monnieri plant extract was taken in a test tube, along with two drops of 10% concentrated sulphuric acid was added and observed for brown colour. Formation of characteristic the brown colour precipitate indicates the presence of steroids.

vii. Test for anthraquinones:
1 ml of plant extract was taken in a test tube along with aqueous ammonia solution was added and observed for colour changes in a test tube. Appearing of pink colour in aqueous layer confirmed the presence of anthraquinones.

viii. Test for phenols:
1 ml crude plant extract was taken in a test tube. After that 3 ml of 10% lead acetate solution was added. The bulk white colour precipitate formed at the surface indicates the presence of phenolic compounds.

ix. Cardiac glycosides:
1 ml of plant extract was taken in a test tube, along with 1 ml of glacial acetic acid, 1 ml of ferric chloride few drops of concentrated sulphuric acid was added. The appearance of reddish brown at the junction of the 2 liquid layers indicates presence of cardiac glycosides.

tax. Coumarin:
2 ml of 10% sodium hydroxide was added to 2 ml of plant extract. Formation of yellow colour in the test tube indicates presence of coumarins.

xi. Triterpenes:
2 ml of methanolic leaf extract was taken along with few drops concentrated sulphuric acid was added. The formation of reddish brown ring in a test tube indicates the presence of Triterpenes.

xii. Quinines:
1 ml of plant extract was taken in a test tube, along with 1 ml of 1% sodium hydroxide were added and mixed well. The appearance of dark blue green indicates confirmed the presence of quinines.

D. FTIR analysis
Functional groups of phytochemicals present in the plant were identified by using the powerful tool FTIR. The FTIR model Jasco was used in this study. For this study 5 mg of dried fine plant powder was taken and mixed with 50 mg of kbr to make a pellet. This pellet was loaded in the FTIR and scan at room temperature in the spectral ranges from 400-4000 cm⁻¹. The results obtained from the FTIR were interpreted by analysed the chemical bonds for particular peak value.

E. TLC
5 gram dried leaf powder of Bacopa monnieri was taken in a conical flask. Along with 10 ml of methanol was added. This setup was kept in magnetic stirrer for 1 day to obtain complete dissolving of phytocompounds of leaf in the methanol solvent. The extract obtained from this maceration process was filtered through No.1. Whatman filter paper. The appropriate solvent system chosen for the present study was composed of methanol: chloroform: hexane (14:4:2). The silica gel precoated readymade aluminum plate obtained from Harish Scientific Company in Vellore used as a stationary phase. Thereafter 5 µl of sample was spotted on the marked place on TLC plate and allowed to run in a TLC chamber saturated with the mobile phase of solvents. Then the spot of antioxidant activity was qualitatively screened by dipping the TLC plates down for 10 seconds in 0.5 mm in DPPH solution. The chromatogram of TLC turned into purple colour due to the presence of antioxidant activity.

F. Antioxidant assays
i. Determination of DPPH scavenging assay:
The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging antioxidant activity of plant extract was determined by the method followed Blois [19]. An aliquot amount of 0.5 ml methanolic leaf extract of Bacopa monnieri was taken in a test tube, along with 2.5 ml of 0.5 mm methanolic solution of DPPH was added. The sample mixture was shaken vigorously and incubated for 30 min in the dark place at room temperature. Thereafter the colour of the solution was measured using UV spectrophotometer at 517 nm (triplicate values). The ascorbic acid was used for a positive control. The scavenging activity of the plant extract was calculated by using the following formula and expressed in a unit % of inhibition.

\[
\text{% of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

ii. FRAP assay
The ferric reducing power of Bacopa monnieri methanolic leaf extract were determined by using FRAP method [20-21]. This reaction is based on the reduction of colorless ferric complex (Fe³⁺ tripyridyltriazine) to blue-colored ferrous complex (Fe²⁺ tripyridyltriazine) by the action of electron donating antioxidants at low pH. The reduction was monitored by measuring the change of absorbance at 593 nm. The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-striaizine) in 40m Mm HCl and with 1
volume of 20 mM ferric chloride. All the required solutions were freshly prepared just before use. 100 µL of samples (mg/mL) were added to 3mL of prepared FRAP reagent. The reaction mixture was incubated in a water bath for 30min at 37°C. The standard curve of ferric chloride (125 µmol, 250 µmol, 500 µmol, 750 µmol and 1000 µmol) was prepared using by same procedure. Then, the absorbance of the samples was measured at 593 nm. The difference between absorbance of sample and the absorbance of blank were calculated to determine the FRAP value. FRAP value was expressed in terms of Mmol Fe2+/g of sample using ferric chloride standard curve Y = 1.7057x – 0.2211, R² = 0.9904.

iii. Nitric oxide radical scavenging activity
The nitric oxide radical scavenging was measured by Griess reaction [22]. For this experiment, the reaction mixture (3ml) containing sodium nitroprusside 10mM in phosphate buffered saline was mixed with various concentrations of methanolic plant extract and incubated at 25°C for 2.5 hours. After the completion of incubation, 0.5ml aliquot amount of Griess reagent [1% sulfanilamide, 2% H3PO4 and 0.1% N- (1-naphthyl) ethylene diamine dihydrochloride] was added. Finally, the chromophore formed in the reaction was measured immediately at 546nm. The inhibition of nitrate formation by the plant extract and the standard antioxidant ascorbic acid were calculated relative to the nitric oxide radical control. The experiment was triplicated and the percentage of scavenging activity was estimated with Curcumin standard reference.

G. Cytotoxicity of methanol leaf extract of Bacopa monnieri on Colon cancer cell line-HCT15
Cytotoxicity analysis was done by the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazoliumbromide) assay method. Working principles of this method is based on the conversion of MTT into formazan crystals by living cells contain NAD(P)H-dependent oxidorecatase enzyme. This assay technology has been widely adopted and remains popular in academic labs to find homogeneous cell viability. The MTT substrate is prepared in a physiologically balanced solution, which has added to culture at a final concentration of 0.2-0.5mg/ml, and incubated for 1 to 4 hours. The formazan crystals formed in the cell culture were then dissolved using solubility solution and absorbed at 570 nm using a plate reading spectrophotometer. The darker solution means the greater the viable and metabolically active cells. The Colon cancer cell line (HCT15) was placed in 96 well plates containing DMEM media with 1X Antibiotic Antimycotic solution and 10% fetal bovine serum (Himedia, India). Along with different concentration of methanol leaf extract of Bacopa monnieri were added. Before that, the concentration of cells was determined to 1×10⁵ cells/well. Then the cell line was incubated in CO₂ incubator at 37° with 5% CO₂. After incubation, the cell line was washed with 200µl of 1X PBS, and MTT reagents were added to each well. The MTT reagent 5 ml containing 5 vials purchase from Sigma Company was used for this experiment. In the present study, this MTT assay method was used to measure the cytotoxicity of plant extract on necrosis of cancer cells in colon.

III. RESULTS & DISCUSSION
The extractive value for Bacopa monnieri leaf was estimated using different solvents and the obtained results were recorded in Table-2 and depicted in Fig.1 (a and b) which showed that the highest yield of extractive value obtained for both methanol (4.9gm) and water (3.6gm) compared all six solvents. The extractive values of other solvents were observed following sequences ethanol (2.2gm) > ethyl acetate (2gm) > chloroform (0.9gm) > hexane (1.7gm) > petroleum ether (0.6gm). Hence the high extractive methanol solvent was selected to extract the phytocompounds from Bacopa monnieri. This result showed a concordance with the findings suggested by [23] that the solvents methanol and acetone appeared to be more effective in extracting bioactive compounds from Mentha viridis. 50% acetone is an ideal solvent to extract the phenol from Salacia chinensis [24].

Plant based antioxidants play a vital role even in trace amount, is capable of preventing of delaying the oxidation of easily oxidizable materials and emerged as a potential therapeutic to prevent free radical generated damage in the human body [25], flavonoids and phenolic [26]. It acts as reductants and inactivators of oxidants [27]. Most of the plants traditionally used to treat various because of having rich antioxidant which are in the form of phytocompounds such as tannins, flavonoids, alkaloids, steroids, etc. Flavonoids present in dietary sources such as tea, red wine, apple tomato, cherry, anion, thyme, parsley, soya-beans and other legumes in the form of flavonols, flavones, isoflavones and flavonones [28]. The preliminary qualitative phytochemical analysis of leaf of methanol extract of plant Bacopa monnieri has been exhibited the presence of various bioactive compounds such as tannins, saponins, alkaloids, steroids, flavonoids proteins, cardioglycolysis and triterpens (Table-1) as similar as with the qualitative analysis of the plant Ephedra intermedia showed the phytocompounds cardioglycocides, alkaloids, reducing sugars, flavonoids, phenols (Ethanol, methanol) [29], phenols and saponins in the methanol extracts of leaves and flowers; alkaloids, flavonoids, terpinoids, carbohydrates, protein and amino acids are present in methanolic extract of leaves, roots and flowers of Bacopa monnieri.
Moringa concansis [30], steroids, alkaloids, cyanide-I
mild concentrations in Sena minosoides. Saponin,
phenols, flavonoids, terpenoids, soluble carbohydrate and
tannins [31]; carbohydrate, amino acids, phenols and
alkaloids in the flowers of Bauhina acuminata [32];
alkaloids, terpenoids, steroids, flavonoids, polyphenols,
glycosides, tannin and saponins in Acanthopora spicifera
and Sargasum wightii [33]; alkaloids, terpenoids, steroid,
tannins, flavonoids, phenols, coumarins, quinones and
glycosides in the brown seaweed Dictota dichotoma [34];
tannins, saponins, flavonoids, cardioligicdes and
alkaloids in the crude extract of Ephedra altissima
aquous and extract of leaf of Ephedra alissima [35];
cardioglycides, alkaloids in the stem and fruit,
flavonoids present in the leaf of Cissus quadrangularis
[36]. After confirmation, the free radical scavenging
ability of this plant leaves were determined by observing
DPPH, FRAP. Nitric oxide scavenging activities. Even
though a number of methods have been proposed to
determine the antioxidant activity, in the present
investigation a widely used method [37-38] is used to
evaluate the free radical scavenging activity and
antioxidant activities of plant extracts.

The leaf extract of Bacopa monnieri has the ability to
scavenge 72.38% of DPPH at 250mg concentration as
similar as exhibited (71.18%) in methanol extracts of the
stem of C. africana (89.69%) and leaves (64.95%) at 0-1
μg/ml and 82.58 % of activity at 500 μg/ml [39]. The
overall observation of DPPH scavenging activities in the
leaf powder of Bacopa monnieri has an increasing trend
when concentration is increase. Therefore, the free
radical scavenging activities of Bacopa monnieri leaf
directly proportionate to their concentration. Nitric oxide
(NO) is an important chemical mediator generated by
endothelial cells, macrophages, neurons, etc. which is
involved in the regulation of various physiological
processes [40]. NO is generated in biological tissues by
specific nitric oxide synthesis (NOSs), which metabolizes
arginine to citralline with the formation of NO via a five
electron oxidative reaction [41]. Nitric oxide or reactive
nitrogen species, formed during their reaction with
oxygen or with superoxides, such as NO2, N2O3, NO3,
NO and NO2 are very reactive. These compounds are
responsible for altering the structural and functional
behavior of many cellular components. The experimention
on scavenging activity of phytochemicals of Bacopa monnieri is very important
because of its neuroprotection activities. Fresh leaf extract of Ixora coccinea, showed 49.43% and 72.18%
NO scavenging, dry flower extract showed 59.43% and
66.93% of NO inhibition and fresh flower has 69.45%
and 77.24% of NO inhibition. FRAP assay was used by
several authors for the assessment of antioxidant activity of various food product samples [42-43]. Like DPPH and

Nitric oxide, the same trend of FRAP scavenging activities was observed (Ferric reducing ability of
plasma), (Table-3 Fig.2(a, b and c). This results showed
some similarity with the antioxidant activities of five
Salvia species such as Salvia multiculalis, Salvia verticellata, Salvia lechnecelyx, Salvia mirzayanii and
Salvia macrosiphon [44].

The FTIR analysis made on methanol, ethanol, hexane,
chloroform and petroleum ether leaf extract of Bacopa
monnieri have 11, 18, 12, 14 and 19 peaks respectively.
The extraction of leaf of Bacopa monnieri in all solvents
such as ethanol, hexane, chloroform and petroleum ether
except the methanol showed bands at 343.50(ethanol),
433.50(hexane), 420.56(Chloroform) and 407.62, 72.32
and 549.96(petroleum ether) cm⁻¹ with C=O stretching
indicate the presence of halo compounds. The band at
572.60 (methanol), 769.94(ethanol), 608.19(chloroform,)
653.48 and 889.64(petroleum ether) would be due to C–
Br stretch, C–H bend indicate the presence of Alkyl
halides and Alkynes. The bands absorbed at
931.70(methanol), 902.58(ethanol), 909.05(hexane),
931.70(Chloroform) and 993.17(petroleum ether) due to
O–H band of carboxylic acids. 1122.57(methanol),
1035.22, 1187.27 and 1264.91(ethanol), 1077.28 and
1287.56(hexane), 1064.34 and 1196.98(Chloroform) and
1109.63(petroleum ether) due to C–N stretch indicate
the presence of aliphatic amines. 1407.26 and
1501.07(methanol), 1339.32, 1452.55 and
1530.19(ethanol), 1420.20 and 1497.84(hexane),
1309.26, 1393.31 and 1501.07(Chloroform) and 1326.38,
1404.02 and 1478.43(petroleum ether) due to N–O
symmetric stretch, C–C stretch (in–ring) indicate the
presence of Nitro compounds, aromatics. 1653.12(methanol),
1624.01(ethanol), 1633.71(Chloroform) and 1649.89(petroleum ether) due to
N–H bend indicate the presence of amines. 1824.58(methanol), 1701.65 and 1841.88(ethanol),
1782.53(hexane), 1824.58(Chloroform) and
1772.82(petroleum ether) due to C=O bend indicate
the presence of Esters, Anhydrides. 2034.86(methanol)
due to C≡C Stretch indicate the presence of Alkynes
(monosubst). 2856.57(methanol), 2914.80(ethanol),
2882.45 and 2940.69(hexane), 2924.51(Chloroform) and
2798.34, 2843.63 and 2930.98(petroleum ether) due to
CH3 and CH2, C–H stretch indicate the presence of
Alkanes, Aliphatic compounds. 3086.26(ethanol),
3015.09(hexane), 3002.15(Chloroform) and
3015.09(petroleum ether) due to =C–H stretch indicate
the presence of Alkenes. 3456.57(methanol), 3429.18
and 3464.77(ethanol), 3358.01(hexane),
3419.48(Chloroform) and 3432.42(petroleum ether) due to
N–H stretch, O–H stretch indicate the presence of
Amines or Alcohols. 3875.62(methanol), 3940.33 and
3998.56(ethanol),

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3742.99 (Chloroform) and 3742.99 and 3979.15 (petroleum ether) due to -OH stretch indicate the presence of Alcohols and Phenols. The screening of plant extract for cytotoxicity on colon cancer cell line of In-vitro study showed a significant level of decline in cell viability (Table 4 and Fig 3). These declining trends were increasing in increasing concentration. This means the death of colon cancer cells are increasing with increasing concentration of plant extract which indicate the leaf extract of Bacopa monnieri possess potent anticancer activities. The present study results showed a strong affinity with Rosa anina extract exhibited a selective cytotoxic effect on colon cancer cells compared with normal colon cells. The extract induced cell cycle arrest at the S-phase and apoptosis via reduced MMP in WiDr cells. Rosa canina extract significantly repressed telomerase expressions at treatment times of 48 and 72 h in WiDr cells [45]. Aerial, leaf and stem extracts of plant Alternanthera sessilis greatly suppressed the growth of colon cancer cells in time and dosage-dependent manner. The cytotoxicity results were rationalized with clonogenic, cell motility and AO/PI assay, where the leaf extract showed the most active activity compared to aerial and stem extracts, [46]. The ethanolic root extract of Euphorbia tehranica showed a significant ctotoxic effect against Caco-2-cell line (p≤0.05). The viability of Caco-2 cells reduced with the dose and time dependent manner [47].

Table 1. List of phytochemicals present in solvent extract of Bacopa monnieri

| S.No | Phytochemical tests | Methanol |
|------|---------------------|----------|
| 1.   | Tannins             | +        |
| 2.   | Saponins            | +        |
| 3.   | Phenols             | -        |
| 4.   | Alkaloids           | +        |
| 5.   | Coumarin            | -        |
| 6.   | Steroids            | +        |
| 7.   | Anthraquinones      | -        |
| 8.   | Flavonoids          | +        |
| 9.   | Cardio glycosides   | +        |
| 10.  | Proteins            | +        |
| 11.  | Triterpenes         | +        |
| 12.  | Quinines            | -        |

Table 2. The extractive value of using different solvents and their dry weight Bacopa monnieri

| Solvents      | Dry weight of raw leaf powder | % of extractive value |
|---------------|------------------------------|-----------------------|
| Water         | 1                            | 3.6                   |
| Methanol      | 1                            | 4.9                   |
| Ethanol       | 1                            | 2.2                   |
| Petroleum ether | 1                        | 0.6                   |
| Chloroform    | 1                            | 0.9                   |
| Hexane        | 1                            | 1.7                   |
| Ethyl acetate | 1                            | 2                     |

Table 3. Free radical scavenging activities of Bacopa monnieri at different concentration

| Tested concentration(µg/ml) | DPPH   | FRAP   | Nitric oxide |
|-----------------------------|--------|--------|--------------|
| 50                          | 23.89±0.81 | 34.76±0.17 | 12.31±0.81 |
| 100                         | 39.47±0.5 | 40.05±0.02 | 27.45±0.98 |
| 150                         | 59.98±0.69 | 42.43±0.06 | 41.62±0.98 |
| 200                         | 66.16±0.50 | 46.78±0.07 | 62.09±1.31 |
| 250                         | 72.38±0.56 | 51.50±0.02 | 73.42±0.82 |

Table 4. Colon cancer cell line different concentration of Bacopa monnieri

| Tested concentration(µg/ml) | % of cell viability |
|-----------------------------|---------------------|
| 25                          | 97.74 ± 2.25        |
| 50                          | 90.21 ± 2.33        |
| 100                         | 79.57 ± 1.59        |
| 250                         | 67.63 ± 3.18        |
| 500                         | 53.33 ± 3.42        |
| Control                     | 100                 |

Table 5. FTIR analysis of the plant Bacopa monnieri in different solvents
IV. CONCLUSION

The present study was concluded that the methanolic plant extract of *Bacopa monnieri* having a high potential free radical scavenging and anticancer activity due to the presence of phytochemical contents such as alkaloids,
terpenoids, steroid, tannins, flavonoids, phenols, coumarins, quinones and glycosides.

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