INTRODUCTION

For the treatment of human diseases, the natural products like plants, animals, minerals are being used. Right from the existence of human civilization, the medical science had been performed practically. The fate of natural ingredients in drug discovery will be more holistic, personalized and involve proper benefit of primal and current therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community [1]. Ayurveda is still one of the most primitive and most practised worldwide and has a good philosophical and practical basis [2, 3]. Recently some work in drug development relates to species of Picrorhiza (hepatoprotective), Commiphora (used as a hypolipidemic agent), Bacopa (memory booster), Asclepias (cardiotoxic) and Carcuma (anti-inflammatory) [4, 5].

B. monnieri (Linn), commonly named as "Brahmi," belongs to the family Scrophulariaceae is an Indian valuable herb [6]. It has been used in Ayurvedic system of medicine traditionally to treat conditions such as fever, inflammation, asthma, epilepsy, pain and memory retardation [7]. As far as the therapeutic importance of B. monnieri in indigenous systems of medicine was found, chemical examinations of the wide variety of plants have also been carried out by many researchers. Some basic investigations reported the separation of the 'brahmine' (alkaloid) from B. monnieri followed by identification of some other alkaloids including nicotine and herpestine [8], D-mannitol, saponin, hersaponin and potassium salts [9]. The memory enhancing activity of B. monnieri has been attributed to the presence of two major saponins bacoside A and B [10, 11]. Some major phytocompounds reported in this plant include phenylethanoid glycosides, amino acids such as σ-alanine, betulinic acid aspartic acid, glutamic acid, and betulinic acid, stigmastanol, β-sitosterol, flavonoids and stigmastanol [12]. Steroidal saponins and Bacosides A and B are the active chemical constituents that are responsible for enhancing both learning and memory [8, 13-15]. Some of the chemical elements of B. monnieri are lipophilic. This means that they can integrate with or dissolve in lipids providing them the ability to pass the blood-brain barrier [16, 17].

Antioxidants had the property to prevent oxidative damage by free radicals that are amenable for variety of human diseases like hypertension, arthrosisclerosis, gastritis, Diabetes mellitus, arthritis, ischemia, AIDS and Alzheimer's disease [18]. Bacosides are known to feed on various free radicals such as peroxides, superoxides and hydroxyl radicals [19, 20]. Previously, antioxidant activity of alcoholic and the hexane extract of B. monnieri on lipid peroxidation by cumene hydro peroxide and ferrous sulphate in the homogenate of rat liver is documented [21].

It is documented that different mechanisms involved in the neuroprotective and memory boosting effects of B. monnieri, such as the attraction and detoxification of metal ions [22, 23], free radical scavenging [24], or improving antioxidant activity have gained much attraction [25]. Animal models have depicted that B. monnieri can employ vasorelaxant, adaptogenic, anti-inflammatory, chelation of metal ions, and cholinergic modulatory effects [26-30]. B. monnieri also shows to inhibit numerous β-amyloid oxidative stress pathways responsible in AD pathology and antioxidant properties related to GSH redox state [31]. Modern studies on B. monnieri adumbrate that its treatment may cause reversible inhibition of spermatogenesis and fertility [32].

As stress is a cause in many diseases, research on a productive anti-stress agent isolated from plants has gained much concern. Researchers have estimated the effect of B. monnieri extract against acute stress and consistent stress models in rats. Various studies on B. monnieri proved its antioxidant effects with metal ion reduction, free scavenging and super oxide dismutase activities and they might be the root of treatment for oxidative stressed diseases including asthma, epilepsy, insanity, inflammation and Cyclic vomiting syndrome diseases [33]. Propanoic and ethanolic extracts of B.
Bacillus subtilis showed antimicrobial activity conversely against various bacterial etiological agents such as Escherichia coli, Streptococcus, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa [34-36]. The phytochemicals such as betulinic acid, wogonin and oroxindin isolated from the aerial parts of B. mongnieri showed aphoristic antifungal activity against Alternaria alternata and Fusarium fujiformis [37].

The Indian herb B. mongnieri may be used as a dietary antioxidant, with certain modes of action to prevent the brain system against oxidative damage and age-related cognitive reduction [38-40]. Current study aimed to identify the phytochemical ingredients of B. mongnieri extracts followed by the evaluation of in vitro antioxidant, anti-inflammatory, antibacterial and antifungal properties of the aqueous and methanolic extract of B. mongnieri.

**MATERIALS AND METHODS**

**Plant material**

The whole plant of B. mongnieri was obtained from the local Ayurvedic clinic, Rampur, Uttar Pradesh. The plant material was identified by the Faculty of Science, Jamia Hamdard, New Delhi (JH/HG/21/2016).

**Chemicals and reagents**

All solvents and chemicals (analytical grade) used for antioxidant, anti-inflammatory, antibacterial and antifungal assay were purchased from Merck and Himedia. DPPH, TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) were purchased from Sigma-Aldrich.

**Microorganisms**

The bacterial strains investigated are identified strains and were obtained from NCCS, Pune, India. The studied bacterial strains include Staphylococcus aureus (MTCC 902), E. coli (MTCC 443), Bacillus subtilis (MTCC 736), Pseudomonas aeruginosa (MTCC 2453). All the test bacterial stock cultures were maintained at 4°C on nutrient agar slants.

**Preparations and extraction procedures of B. mongnieri aqueous and methanolic extract**

The aqueous and methanolic extract of B. mongnieri was prepared by dissolving 1 g of B. mongnieri in 20 ml of solvents i.e. distilled water (DW) and methanol respectively. The sample was soaked for 24-48 h for the complete extraction of all important phytochemical constituents. Further, it was strained through muslin cloth. The samples were then centrifuged and the supernatants were collected. The supernatant served as B. mongnieri aqueous extract (BmAЕ) and B. mongnieri methanolic extract (BmME) for the further evaluation.

**Identification of phytochemicals from BmAЕ and BmME**

The extracts BmAЕ and BmME were qualitatively tested to identify the presence of phytochemicals such as alkaloids, phenols, amino acids, flavonoids, saponins, tannins, quinones, carbohydrates, glycosides, steroids, carboxylic acids, resins, proteins and terpenoids according to the method described by [58].

**Evaluation of antioxidant potential of BmAЕ and BmME**

**DPH assay**

BmAЕ, BmME and the standard ascorbic acid were checked for their antioxidant potential on the basis of free radical scavenging effect of stable DPPH according to the protocol of Gouveas and Abraham. With minor modifications [41]. A range of diluted working solutions of the BmAЕ and BmME were prepared in distilled water and methanol respectively. The aqueous solution of ascorbic acid (1 mg/ml) was also prepared for the comparative assay. DPPH (0.1 mM) was prepared in 80% methanol and 500µl of this solution was mixed with 500µl of serially diluted sample and standard ascorbic acid solutions separately. The obtained reaction mixtures were kept in dark for 15-30 min and absorbance was recorded at 517 nm spectrophotometrically. DPPH solution was used as control and the range of diluted BmAЕ and BmME was taken as blank. The absorbance was recorded and DPPH scavenging was calculated using the formula given below:

\[
\text{DPPH scavenging Activity (\%)} = \left[ \frac{A_{ac-At}}{A_{Ac}} \right] \times 100
\]

Where Ac and At represent absorbance of control and test sample respectively.

**Superoxide dismutase (SOD) assay**

SOD assay was performed according to the method of Kakkar et al. with minor modification [42]. The assay mixture contained 0.2 ml of BmAЕ and BmME in water and methanol, 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of Phenazine methosulfate (PMS), and 0.3 ml of Nitro blue tetrazolium chloride (NBT) in a total volume of 1.8 ml. The reaction was initiated by the addition of 0.2 ml of Nicotinamide Adenine Dinucleotide (NADH). The assay mixture was incubated at 30°C for 5-10 min and glacial acetic acid was added for termination of the reaction. 4.0 ml n-butanol was also added to the reaction mixture and allowed to stand for 10-20 min at room temperature and then centrifuged. The intensity of the chromogen developed in layer of butanol was recorded at 560 nm. One unit of enzyme activity is defined as the amount of extract that gave 50% inhibition of NBT reduction in one minute. SOD assay is based on inhibition of formation of NADH-PMS-nitroblue tetrazolium formazan. The colour formed at the end of reaction can be extracted into butanol and measured at 560 nm.

**Catalase assay**

Catalase activity was assayed following the method of Jambunanath et al. with minor modification [43]. H2O2-Phosphate buffer (3.0 ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of BmAЕ and BmME and mixed thoroughly. The time required for a drop in absorbance by 0.05 units was noted at 240 nm. The H2O2-Phosphate buffer was served as control. One enzyme unit was calculated as the amount of enzyme present in extracts required to decrease the absorbance at 240 nm by 0.05 units. The UV absorption of H2O2 can be measured at 240 nm, whose absorbance decreases when degraded by the enzyme catale. From the decrease in absorbance, the activity of enzyme found in extracts can be calculated.

**Reduced glutathione (GSH) assay**

Reduced glutathione (GSH) was determined by the method of Moron et al. with minor modification [44]. 0.2 ml of BmAЕ and BmME of different concentration was taken and volume was maintained up to 1.0 ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH was also prepared to concentrations ranging from 10-20 nmoles. 2.0 ml of freshly prepared 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) solution was added to the reaction mixture. After 10 min, the intensity of the developed yellow colour was measured in a spectrophotometer at 412 nm. The results were expressed in µM/min/µl of both the extracts.

**Ferric reducing antioxidant power (FRAP) assay**

The antioxidant potential of BmAЕ and BmME was assessed based on the Ferric Reducing Antioxidant Power (FRAP) assay by the method of Sudha et al. with minor modification [45]. A total of 75 µl of BmAЕ and BmME and 225 µl of DW and methanol respectively were added to 1.5 ml of freshly prepared FRAP reagent [10 parts of 300 mmol sodium acetate buffer of pH 3.6, 1 part of 10 mmol 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) solution and 1 part of 20 mmol FeSO4.7H2O]. The reaction mixture was incubated in the dark for 4 min. The increase in absorbance with the formation of colored product (ferrous tripriyidyltriazine complex) was recorded at 593 nm. FRAP working solution was taken as blank. The antioxidant capacity of the BmAЕ and BmME was determined based on a calibration curve plotted using FeSO4.7H2O at a concentration ranging between 0.125 and 2 mmol taking the concentration of FeSO4.7H2O mmol on x-axis and the absorbance at 593 nm on y-axis. Results were expressed in mmol of Fe (II)/g of extract. The relative activity of the extracts was compared to standard ascorbic acid and analysed in parallel. Absorbance of each diluted extract and standard were expressed in mmol of FRAP value.

**Evaluation of anti-inflammatory activity**

**Inhibition of albumin denaturation**

The anti-inflammatory assay was done according to the method of Mizushima et al. with minor changes [46]. A range of diluted
working solutions of the BmAE and BmME were prepared and mixed with 1% aqueous solution of bovine serum albumin (BSA) fraction. The reaction mixtures were incubated at 37 °C for 20 min and then heated at 57 °C for 20 min. 1% BSA was taken as control and water was taken as blank. Aspirin (100μg/ml) was taken as standard drug. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Percent inhibition of protein denaturation was calculated using formula:

\[
\text{Percentage inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

### Proteinase inhibitory action

The test was performed according to the modified method of Oyedepo et al. [47]. 0.06 mg trypsin was added to the reaction mixture containing 1 ml of 20 mmol Tris-HCl buffer (pH 7.4) and 1 ml of diluted sample of BmAE and BmME with water and methanol respectively. The assay mixture was incubated at 37 °C for 10-15 min and then 1 ml of 1% (w/v) casein was added. The assay mixture was further incubated for an additional 20 min. 70% perchloric acid was added to the mixture in order to arrest the reaction. Precipitated protein suspension was then centrifuged and the absorbance of the supernatant was read at 210 nm. The inhibition of proteinase inhibitory activity in percentage was calculated.

\[
\text{Percentage proteinase inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

### Evaluation of antimicrobial activity

#### Determination of antibacterial activity

Antimicrobial activity of the BmAE and BmME was tested against four bacterial strains (Pseudomonas aeroginosa, Bacillus subtilis, Staphylococcus aureus and Escherichia coli) as per the method of Barbade and Datar with few modifications [45]. Overnight cultures were prepared in Luria broth (LB) media by inoculating a single colony from agar plates and incubated at 37 °C for 12 h. Overnight cultures were diluted with fresh LB media to approximately 10^6 colony forming units (CFU) and incubated at 37 °C for 12-14 h in the presence of BmAE and BmME compared to the growth of the control culture where only media and bacterial inoculums was present. Experiment was repeated twice for the confirmation. The absorbance was recorded at 600 nm. The percentage inhibition was calculated by using the formula:

\[
\text{Percentage Inhibition} (%) = \left( \frac{dc-dt}{dc} \right) \times 100
\]

Where dc and dt represent the absorbance of control and treated sample strains respectively.

#### Determination of antifungal activity

The minimum inhibitory concentration of BmAE and BmME against Candida cells (ATCC 10261 and ATCC 90028) was determined by broth dilution method. Cultures were grown with or without test compounds in the media. Two fold dilutions of the test compound were carried out as an initial step. Also, antifungal activity of BmAE and BmME in solid media was determined by the Disc Diffusion Method. Candida cells (10^5 cells/ml) were inoculated in molten YEPD agar (40 °C) and poured into a petriplates. Filter discs were placed on solid agar and different concentrations of test compounds were applied on the disc in 10μl volume. The average diameter of the zone of inhibition was recorded in mm.

### Statistical analysis

The experiments were done in triplicates. Results were expressed as graphs representing means±SEM (Standard Error of Mean) using the software Graph Pad Prism 5.0.

#### RESULTS

#### Identification of phytocompounds from BmAE and BmME

Qualitative phytochemical analysis of aqueous and methanolic extracts of B. monnieri plant revealed the presence of phenolics and non-phenolics phytocompounds such as total phenols, tannins and flavonoids, alkaloids, sterol, resins, terpenoids, xanthoproteins, quinones, glycosides and saponins while the steroids and carboxylic acids were absent in both type of extracts. Other compounds were found in fairly detectable quantity (table 1).

### Table 1: Phytochemical composition of methanolic and aqueous extracts of B. monnieri

| Phytochemical constituents | BmAE | BmME |
|---------------------------|------|------|
| Alkaloids                 | +    | +    |
| Flavonoids                | +    | +    |
| Steroids                  | -    | -    |
| Saponins                  | +    | +    |
| Phenols                   | +    | +    |
| Resins                    | +    | +    |
| Tannins                   | +    | +    |
| Terpenoids                | +    | +    |
| Xanthoproteins            | +    | +    |
| Quinones                  | +    | +    |
| Glycosides                | +    | +    |
| Carboxylic acid           | -    | -    |

Evaluation of antioxidant potential of BmAE and BmME

### DPPH assay

The decrease in the absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as the colour changes from purple to yellow. B. monnieri showed DPPH radical scavenging activity in a concentration-dependent manner as shown in the fig. 1. The BmAE and BmME showed DPPH radical scavenging activity in a concentration-dependent fashion with the highest and lowest scavenging at amount 500 μl and 50 μl of the extracts. BmAE demonstrated highest % DPPH radical scavenging of 94.19316±0.0047% and lowest of 17.18339±0.0059%. While the BmME showed highest %DPPH radical scavenging of 94.7942±0.0050% and minimum of 24.47114±0.0052%. The results were quite comparable to that of standard i.e. ascorbic acid with maximum % DPPH scavenging of 99.67775±0.0053% and minimum of 61.811 49±0.0046%.

![Fig. 1: 1, 1-diphenyl-2-picrylhydrazyl free radical scavenging activity of methanolic and aqueous extract of B. monnieri were calculated and compared to ascorbic acid i.e. standard.](image)

### SOD assay

SOD molecules are known to convert superoxide free radicals to hydrogen peroxide and molecular oxygen. It is a major defense for aerobic cells in combating the toxic effects of superoxide radicals. They belong to a large family of isoenzymes that mediate cellular response to oxidative stress and represent the main enzymatic source of peroxides. The degradation of superoxide radicals per minute was evaluated and this radical was degraded on an average unit of 0.006 by BmAE and 0.023 units by BmME (fig. 2).

### Catalase assay

The UV absorption of hydrogen peroxide can be measured at 240 nm, whose absorption decreases when degraded by the enzyme catalase.
From the decrease in absorbance, the enzyme activity can be portrayed as the concentration of hydrogen peroxide is decreasing significantly in dose-dependent manner. The 100% hydrogen peroxide was degraded to 75.16246 ±0.0063% and 71.58474±0.0067% when initially small amount of BmAE and BmME was added to the cuvette respectively. Degradation per minute observed upto 10 min and finally there was approximately 7.889728±0.0068% and 2.229903±0.0059% radical was remained (fig. 3).

Reduced glutathione assay

Glutathione peroxidase catalyses the reduction of hydrogen peroxides by reduced glutathione (GSH) and functions to protect the cell from oxidative damage. GSH is the important part of non-enzymatic antioxidants and it can remove peroxynitrite (ONOO-) with the formation of oxidized glutathione (GS-SG), which is converted back into GSH by NADPH-dependent glutathione reductase. Reduced glutathione on reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) produces a yellow coloured product that absorbs at 412 nm. DTNB was used as control and the DTNB with different concentration of BmAE and BmME was used as test sample. The concentration of GSH was expressed in µM/min/µl extract and estimated upto 200 µl of both extracts. GSH was found in low concentration at 50 µl and in high concentration at 200 µl volume of both BmAE and BmME (fig. 4).

FRAP assay

This assay measures the ability of antioxidants in the samples to reduce the ferric ions to a coloured ferrous product at 593 nm. The results showed that FRAP value of B. monnieri increased in concentration-dependent manner. The highest absorbance of FRAP was observed in B. monnieri at 500 µl and the lowest was that at 50µl in both BmAE and BmME with a maximum FRAP value 1.0527±0.065 mmol and 1.0067±0.041 mmol and minimum value of 0.3027±0.052 mmol and 0.275±0.057 mmol respectively as compared to standard maxima at 1.1847±0.049 mmol and minimal at 0.373±0.032 mmol respectively. These concentrations were effective to react with ferric tripyridyltriazine (Fe=O-TPTZ) complex and produce a blue coloured ferrous tripyridyltriazine (Fe=O-TPTZ). From the observations, it is clear that B. monnieri showed fair antioxidant activity comparable to ascorbic acid (fig. 5).

Evaluation of Anti-inflammatory activity

Inhibition of albumin denaturation

If there occurs any infection or damage to the body or tissue then body respond against the infection through inflammation. Protein denaturation is one of the major causes of inflammation. Due to this, we have tried to find out the ability of B. monnieri to inhibit protein denaturation. The results showed that BmAE and BmME were effective in inhibiting thermally induced albumin denaturation at different concentrations. The BmAE showed the highest percentage inhibition of albumin denaturation of 44.93771±0.0071% and the lowest of 21.32969±0.0075% while BmME resulted in maximum % inhibition of 39.14707±0.0068% and minimum % inhibition of 15.53905±0.0065% at extract volume or amount of 1000 µl and 100 µl respectively (fig. 6).

Proteinase inhibitory activity

Results exhibited significant anti-protease activity at different concentrations of BmAE and BmME. The BmAE demonstrated the highest percentage protease inhibition of 29.94818±0.0071 % and the lowest of 14.47679±0.0082% while BmME resulted in maximum protease inhibition of 74.337±0.0073% and minimum inhibition of 25.327±0.0079% at extract volume or amount of 1000 µl and 100 µl respectively (fig. 7).
Antifungal activity

Antifungal assay of the *B. monnieri* extract was performed against *Candida* cells (ATCC10261 and ATCC 90028). The extract showed no zone of inhibition up to 1.5 mg/ml as well as there is no inhibition in growth of *Candida* cells up to 1 mg/ml in broth as well. Our results showed that BmAE and BmME showed no potent antifungal activity up to 1 mg/ml, increased concentration of extracts might lead the antifungal effect.

DISCUSSION

*B. monnieri* has been traditionally used in Ayurvedic medicine to treat conditions such as fever, inflammation, pain, asthma, epilepsy, and memory decline [11]. In this study, the phytochemical screening of aqueous and methanolic extract of whole plant of *B. monnieri* revealed that among the phytochemicals, investigated in presence of phenolic (total phenols, tannins and flavonoids), and non-phenolics (alkaloids, sterol, resins, terpenoids, xanthoproteins, quinones, glycosides and saponins) compounds were detected while steroids and carboxylic acid were absent. The presence of some of these secondary metabolites suggests that the plant might be of medicinal importance. The methanolic extract of *B. monnieri* gave more intense result as compared to the aqueous extract. The phytochemical constituents present in *B. monnieri* may be responsible for its therapeutic role and other pharmacological properties, the constituents of which are reported here and detected during the experiments might be use for medicinal purpose. The presence of phenolic compounds (total phenols, tannins and flavonoids) provides pharmacological activities like anti-cancer, anti-oxidant, antimicrobial, and anti-inflammatory [48-53] that may suggest an association to the species here investigated. All the plants exhibited potent antioxidant activity. Tannins and flavonoids are said to be responsible for scavenging free radical species and hence act as a major antioxidant group in plants [54]. The theory of aging skin by the action of free radicals is based on the failure mechanism of natural antioxidant in vivo and in vitro studies explain the relationship of increasing level of ROS due to loss in activity of enzymatic and non-enzymatic agents and aging. Ascorbic acid is one of the most popular natural antioxidant as it oxidizes ascorbate to dehydroascorbate and hence eliminates ROS. It also demonstrates other important physiological functions such as role as cofactor for enzymatic activity of prolyl hydroxylase, which hydroxylates prolyl residues for collagen and elastin [55].

It is common consensus that the cellular aging process can be prevented by plants' phenolic substances, which has motivated the investigation of *B. monnieri* metabolites and its possible action in the prevention of cellular aging. The result depicted similar pattern of antioxidant activity by BmAE, BmME and ascorbic acid (BmME being higher than BmAE) suggesting *B. monnieri* as a potent antioxidant source.

Inflammation is a common phenomenon and it is a reaction of living tissue towards injury. Prostaglandin-E2, an effective vasodilator in combination with histamine and bradykinin which are potent inflammatory vasodilators lead to the increased blood flow and redness in the region of acute inflammation [56]. Tannins are only remarkable for their antiseptic property but also for their astringent action. This astringent property afford them the therapeutic value in arresting haemorrhage by constructing blood vessels and in protecting wounds, inflammation and ulcer from external irritation by the participating surface protein which form impervious coating on them. Thus it is evident that the constituents are sufficient to cure infection and tannins are also responsible to cure inflammatory diseases. The results obtained support the use of *B. monnieri* extract locally against inflammation, ROS related diseases, fever, ulcer, infection etc.

Alkaloids being bitter substance exert notable antimicrobial action. So it is quite reasonable alkaloid containing plants show effectiveness against microbial diseases. The results show that BmAE and BmME both showed antibacterial activity in concentration dependent manner which was quite comparable to that of the standard drug.

**Evaluation of Antimicrobial activity**

**Antibacterial activity**

Antimicrobial assay of the aqueous extract was examined against various bacterial strains by accessing the percentage inhibition in presence of *B. monnieri* compared to the control where only media and cultures were added. The results suggested that BmAE and BmME exhibit bactericidal property in vitro i.e. the growth of microorganisms was inhibited in its presence. It was found that BmAE was most effective against *B. subtilis* with %MGI of 73.91±0.0069% and was least effective against *S. aureus* with %MGI of 52.04±0.0063%. BmME showed maximum %MGI of 79.89±0.0081% against *B. subtilis* whereas least against *S. aureus* i.e. %MGI of 54.78±0.0076%. Whereas, synthetic antibiotic ampicillin showed almost complete inhibition against all the strain at the final concentration of 0.5 mg/ml (fig. 8).

**Antifungal activity**

Antifungal assay of the *B. monnieri* extract was performed against *Candida* cells (ATCC10261 and ATCC 90028). The extract showed no zone of inhibition up to 1.5 mg/ml as well as there is no inhibition in growth of *Candida* cells up to 1 mg/ml in broth as well. Our results showed that BmAE and BmME showed no potent antifungal activity up to 1 mg/ml, increased concentration of extracts might lead the antifungal effect.
CONCLUSION

Aqueous and methanolic extracts from *B. monnieri* was quantified for the main phytocompounds present in extracts. The presence of various phenolics and non-phenolics phytocompounds concluded that the plant might be of medicinal importance. The varying antioxidant (free radical scavenging) activities of extracts when compared to standard antioxidant i.e. Vitamin C, suggested the possibility that the antioxidant activity of this medicinal plant may contribute to play their role against various ROS mediated disorders such as cellular aging and cancer, becoming an alternative in the fight against skin aging and cancer cells. Altogether, these results establish the therapeutic applications of *B. monnieri* and its use as herbal medicine for the prevention of inflammation and treatment of ROS and bacterial diseases. It can also be accomplished as an important mark in the field of human health and sciences. Finally, considering the results obtained, as future perspectives, we intend to evaluate some other biological activities, such as wound-healing, antimalarial, antiviral and anti-cancer activity.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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