IJPSR (2023), Volume 14, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 August 2022; received in revised form, 27 September 2022; accepted, 23 October 2022; published 01 April 2023

IN-VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF WHOLE PLANT EXTRACT OF *BIOPHYTUM* SPECIES

Chitra C. Nair [*1], C. S. Hima [2] and A. Asheeta [3]

Department of Pharmacy Practice [1], Department of Pharmaceutical Chemistry [2], Department of Pharmacology [3], Ezhuthachan College of Pharmaceutical Sciences, Marayamuttom, Neyyattinkara, Thiruvananthapuram - 695124, Kerala, India.

Keywords:

Biophytum reinwardtii, *Biophytum veldkampii*, *in-vitro* antioxidant, Free radical, TPC, TFC

Correspondence to Author:
Chitra C. Nair

Associate Professor,
Department of Pharmacy Practice,
Ezhuthachan College of Pharmaceutical Sciences, Marayamuttom, Neyyattinkara, Thiruvananthapuram - 695124, Kerala, India.

E-mail: chitracnair@gmail.com

ABSTRACT: The whole plant of *Biophytum reinwardtii* (Zucc.) Klotzsch (BR) and *Biophytum veldkampii* A.E.S. Khan & *et al*. (BV) were evaluated for their antioxidant activities, total phenolic content (TPC), and total flavonoid content (TFC). The plants were successfully extracted with varying polarity solvents like pet ether, chloroform, ethyl acetate, and ethanol. Phytochemical evaluation of various extracts revealed the occurrence of alkaloids, carbohydrates, phenolic compounds, tannins, and flavonoids. The TPC was estimated by the Folin-Ciocalteu method and indicated that the maximum amount was present in ethyl acetate extract of BV (61.06 mg of GA/g of extract) followed by BR (60.08 mg of GA/g of extract). The TFC was highest in the ethyl acetate extract of BV (106.67 66 mg of RU/g of extract), followed by BR (96.66 mg of RU/g of extract). In *in-vitro* studies, ethyl acetate extract showed maximum activity. For BR, the IC_{50} value of DPPH radical, Nitric oxide radical, ABTS radical, Superoxide radical, and Hydrogen peroxide radical scavenging activity was found to be 48.95, 60.72, 54.85, 58.53, and 59.23 µg/ml, and for BV it was 49.81, 61.17, 55.45, 58.5 and 60.23 µg/ml respectively. The study outcome reveals that ethyl acetate extract of BR and BV has substantial free radical scavenging and antioxidant potential that may be ascribed to bioactive phytochemicals like amentoflavone present in them.

INTRODUCTION: Free radicals constitute the primary cause of many disorders. In normal metabolism, Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are created in the body. They are well-known for performing a double role as harmful and helpful species equally [1]. Oxidation can destroy many of the cell's vital components when made in excess. Fats, proteins, polynucleotides, and carbohydrates are a few of the biological macromolecules that can interact with ROS.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(4).1828-37

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(4).1828-37

Oxidative stress arises when free radicals from oxygen pass on beyond the cell's capacity to quench free radicals [2]. Excessive emergence of radicals damages biological material and is the main episode in the origin of various diseases like diabetes, cancer, cardiovascular disease, inflammation, hepatic, renal and neurodegenerative disease [3, 4]. Antioxidant defense processes in healthy organisms roughly balance the origination of these reactive species [5].

Antioxidants are vital in terminating free radical mediated reactions by hunting them and thereby keeping the individual free from its detrimental effects [6]. Herbs are vital remedial supports for decreasing diseases in mankind. It's a distinguished fact that plants contain plenty of antioxidants that negate cellular damage due to oxidative stress. Evaluation of plants for substances with

pharmacological activity has steered to the innovation of novel agents with competent action against many illnesses [7]. It has stood proven that phytochemical compounds could stop free radical-facilitated effects in living beings. So, several related ailments owing to free radicals such as cancer and diabetes can be prevented [8].

The genus *Biophytum* is one among the medicinally important herb related to the family oxalidaceae. There are around 70 species of *Biophytum* spread out in the tropic and subtropiczones of biosphere. In India, it is described to have seventeen species; in Kerala, about nine species of this genus are found [9, 10]. In Kerala, plant bloom is well-thought-out among the ten holy flowering herbs called the 'Dasapushpas'. Traditionally this group of flower is provided appropriate significance in Kerala [11].

This genus has been utilized diversely to heal many illnesses. It is a vital therapeutic plant that benefited by tradition and Ayurveda. The different varieties of *Biophytum* are employed in conventional folk treatment for an extended period, showing their therapeutic worth. In Ayurveda, the herb is utilized as energizer, intoxicant, and for the cure of many diseases such as lung diseases, seizures, fibrous xanthoma, joint inflammation, lumbago, osteophyte, osteoarthritis, cervical disc conditions and limb muscle spasm. The herb is applied to cleanse the uterus following labour. The herb is as well utilized for handling substantial blood loss observed in females; hence, in addition, named as "Teendanaazhi" [12, 13].

For the present study, two species of *Biophytum* are considered, namely *Biophytum reinwardtii* (Zucc.) Klotzsch (BR) and *Biophytum veldkampii* A.E.S. Khan & *et. al.* (BV). They are widespread on pavements, unused areas, jungles and the environment with humid loams [14]. BR is an ordinary, annual herb noticed all through the monsoon. It is seen entirely over the moderate hot portions of India. The appearance of the plant is alike a small tree. The herb is dispersed widely in the northern part of India from Uttarakhand to northeastern states, up to a height of 1800 m and spreading into southern parts as well. It is also seen in the Himalayan range, South China, Srilanka, and Malaya [15, 16]. BV appears like a small tree and is innate in India. They are generally seen at low and moderate heights above sea level, in moist areas (typically grasslands) of Asia, Tropical Africa and India, in heavy group of bushes in open areas, meadows and in the shadows of shrubs and trees [17]. The current research aims to determine the *in-vitro* antioxidant and free radical scavenging activity of whole plant extract of *Biophytum* species.

EXPERIMENTAL: BR and BV Fresh plants were collected locally from the Thiruvananthapuram district. The identification and authentication of plant was done by Dr. T.S Swapna, Prof. and Head, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. (Ref. No. KUBH 10882 and No.KUBH 10883)

Preparation of Extract: The collected plants were cleaned with water to eliminate dust and dirt. The plants were set aside to become dry in shadow for three weeks. Then the plants were coarsely powdered, sieved (mesh size was 40), and stored in an air-tight container at room temperature. Powdered plant materials were extracted in Soxhlet apparatus with solvents of varying polarity in a series, i.e. pet ether followed by chloroform, ethyl acetate and ethanol. For BR the percentage yield of pet ether, chloroform, ethyl acetate and ethanol were found be 3.17% w/w, 4.26% w/w, 9.33% w/w and 5.08% w/w, respectively and for BV percentage yield of pet ether, chloroform, ethyl acetate and ethanol were found be 2.85% w/w, 3.92% w/w, 8.94% w/w and 5.69% w/w respectively. Finally extracts were concentrated in a rotary evaporator at decreased pressure at 40-50 °C and ultimately attained a waterless residue [18]. Qualitative phytochemical evaluation of different extracts by standard procedures revealed the presence of carbohydrates, proteins, aminoacids, phenolic compounds, alkaloids, tannins, steroids and flavonoids [19].

Estimation of Total Phenolic Content (TPC): The entire phenolic content of different extracts of BR and BV was examined with Folin–Ciocalteu technique according to method of Singleton and Rossi, 1965 with a minor alteration [20]. 1 ml herb extract was mixed to 5 ml Folin–Ciocalteu's testing agent. After 5 minutes, 1 ml 7.5 %w/v Na_2CO_3 was supplemented, which was held at normal temperature for 30 min and absorbance was determined at 765 nm. Varied Gallic acid

concentrations in ethanol were availed to make a standard curve. The estimation of the sample was done in triplicate. The TPC of the extract was stated as gallic acid equivalents (GAE) in mg/100 ml.

Estimation of Total Flavonoid Content (TFC): TFC in both plant extracts was evaluated by spectrophotometry, explained by Quittier *et al* [21]. BR and BV extracts in 1 mg/ml were mixed with 1ml 2% $AlCl_3$ in ethyl alcohol and let to remain for 60 min at room temperature. Absorbance was computed by spectroscopy at 415 nm. For each test, samples were made in triplet, and the mean value of absorbance was attained. The same process was replicated for standard rutin solution, and calibration graph was made. Based on measured absorbance, flavonoid matter was interpreted from the graph. TFC was stated as mg of RU/g of extract.

***In-vitro* Antioxidant Activity:**

Estimation of DPPH Antioxidant Assay: DPPH radical scavenging activity was estimated based on Blois [22]. Basically, this approach is a decolorization test that measures the diminution in absorbance caused by adding the antioxidant to a DPPH sample in ethanol. Because the radical molecule is stable and not to be formed as in alternative sorts of radical regimes, the DPPH assay is regarded as a valid and simple assay to measure antioxidant hunting activity. Ascorbic acid was used as reference standard and a stock solution was made by dissolving 1mg/1000µl in distilled water. The ethyl acetate extract of BR, BV or standard solution at different concentration were mixed with 1ml of 0.3mM DPPH ethanolic solution.

The mixture was mixed exhaustively, and it was kept at 20-25°C for 30 minutes in a dark area and absorbance was measured at 517 nm. 3 ml ethanol served as blank and 1 ml of 0.3 mM DPPH ethanolic solution without extract or standard served as control. The experimentation was done in triplicate.

Scavenging action was stated as the percentage inhibition calculated with the following formula.

$$\% \text{ Inhibition} = A_c\text{-}A_t / A_c \times 100$$

Where A_c was the absorbance of control and A_t was the absorbance of extract or standard

Estimation of Nitric Oxide Scavenging Assay: Nitric oxide scavenging assay was carried out based on Madan *et al.* [23]. Sodium nitroprusside in water at pH 7.2 spontaneously produces nitric oxide. The so-formed nitric oxide reacts with oxygen and generates nitrite ions that can be measured by Griess reaction. Nitric oxide scavenger's existence results in reduced nitrite ions making as it struggles with oxygen for nitric oxide. Test sample in various concentrations (10-100µL/ml) were mixed with sodium nitroprusside (10mM) dissolved in PBS (pH 7.2). The mixture was kept for 5 hours at 30°C. Then, 0.5ml of the solution was mixed with Griess reagent (made by blending an equivalent amount of 1% sulphanilamide in 2 % H_3PO_4 and 0.1% NEDA in water). The mixture was stirred thoroughly and kept for 5 min to accomplish diazotization reaction. Subsequently, 1ml of NEDA was supplemented and kept in dim light for 30 min. The OD of rose shaded chromophore produced was taken at 540nm. A control sample containing the same volume without any test sample or standard was made, and Ascorbic acid served as the reference standard. The experimentation was executed thrice and % inhibition was measured. Radical foraging activity was worked out by the formula specified underneath:

$$\% \text{ Inhibition} = A_c\text{-}A_t / A_c \times 100$$

Where A_c was absorbance of control and A_t was absorbance of test or standard

Estimation of ABTS Radical Scavenging Assay: The 2, 2-Azinobis-(3-ethylbenzothiazoline - 6 - sulphonic acid (ABTS) test is focused on scavenging of the radical cation ABTS•+ based on the method explained by Re *et al.* [24]. A chemical reaction involving ABTS and potassium persulfate results in the formation of ABTS radical cation. Potassium persulfate oxidizes ABTS to produce the free radial, reduced by hydrogen supplying radical hunters existing in test samples. The magnitude of each extract to bleach the ABTS radical cation was availed to assess its antioxidant capability. An aqueous solution of ABTS and potassium persulfate (1:1) was kept in darkness at 20-25°C for 12-16 hrs prior to usage. This reaction generates ABTS radical cation. Following the addition of different concentrations of sample extract to 0.3 ml

solution of ABTS, the final volume was made up to 1ml using ethanol. The absorbance was determined after 30 minutes of preliminary mixing. A suitable blank was made with all agents except the extract. The whole process was repeated in triplicate. The anti-oxidant activity was compared to Ascorbic acid, which was used as the standard. Absorbance at 745nm was set on to assess percentage inhibition which was established by the succeeding equation:

$$\% \text{ inhibition} = (A_c - A_t) / A_c \times 100$$

Where, A_c was the absorbance of ABTS radical and ethanol and A_t was the absorbance of ABTS radical and test sample/standard

Estimation of Superoxide Radical Scavenging Assay: The super oxide radical scavenging assay was done based on method described by Nishimiki *et al* [25]. The scavenging action for super oxide is quantified relating to hindering its making. The phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system makessuper oxide which reduces Nitro blue tetrazolium (NBT) to lilac formazan. An antioxidant added to this system is capable of interacting with super oxide, inhibiting the making of formazan and subsequent drop in absorbance at 560nm.

Different concentrations of ethyl acetate extract of BR and BV or standard were mixed with NBT solution (1ml 156 µM) in phosphate buffer (100 mM, pH 7.4) and NADH (1 ml 468 µM) in phosphate buffer (100 mM, pH 7.4). The reaction mixture was kept for 5 min and absorbance was determined at 560 nm against a standard solution of ascorbic acid to determine amount of formazan produced. In control, pure DMSO was taken in preference to alkaline DMSO and the reference standard was Ascorbic acid. % inhibition was computed as stated below.

$$\% \text{ inhibition} = A_c - A_t / A_c \times 100$$

Where A_c was absorbance of control and A_t was absorbance of test or standard

Estimation of Hydrogen Peroxide Foraging Assay: The hydrogen peroxide scavenging assay was done based on Ruch *et al.* [26]. 40mM hydrogen peroxide solution was made in PBS of pH 7.4. A stock solution of the test sample in the strength of 10mg/ml was made. From this, various strength of the sample was pooled with 0.6ml of hydrogen peroxide solution. A control having an equal quantity of distilled water but lacking sample was used. The absorbance of hydrogen peroxide at 230nm was measured at zero and ten minutes, versus a blank with phosphate buffer lacking hydrogen peroxide.

$$\% \text{ inhibition} = A_c - A_{t\,/}\, A_c \times 100$$

Where A_c was absorbance of control and A_t was absorbance of test or standard

Statistical Analysis: The study outcomes were specified as mean ± SEM. The data was studied by ANOVA followed by Dunnett's multiple comparison tests. Significant values were outlined as those with a probability value less than 0.05, 0.01 and 0.001.

RESULTS AND DISCUSSION:

Quantitative Estimation of Phenolic Content: The total phenolic content of ethyl acetate extract of BR and BV was expressed in terms of gallic acid equivalents. The phenolic matter was quantified and extended from 7.37 to 60.08 (mg GAE/g) for different extracts of BR and 7.96 to 61.06 for various extracts of BV. Both plants' crude extract comprised a substantial amount of phenolic matter and ethyl acetate bears noticeably more content followed by ethanol, chloroform and pet ether **Table 1**.

Herbal tissues are a plentiful source of phenolic complexes and are availed as antioxidants. This antioxidant action is worthy and effectual for some illnesses, due to the occupancy of hydroxyl groups that play a focal part in their hunting capability. So, they can react with active oxygen radicals like hydroxyl radicals. Phenolics have aromatic features and unveil major antioxidant action. It quenches singlet oxygen, functions as hydrogen donar, reductant [27] and possesses appropriate metal chelation characteristics [28].

Polyphenolics contained in herbal extract interact with a redox agent to make a blue chromophore comprised of a phospho-tungistic-phosphomolybdenum composite, which is quantitated by a UV-visible spectrometer. The strength of absorption depends on the extent of phenolic complexes.

TABLE 1: TOTAL PHENOLIC MATTER IN DIFFERENT EXTRACTS OF BR AND BV

Plant Extract	TPC (µg of Gallic acid equivalent /mg)
BRPE	7.37±0.05
BRCL	8.42±0.26
BREA	60.08±0.12
BRET	20.72±0.07
BVPE	7.96±0.28
BVCL	8.87±0.18
BVEA	61.06±0.15
BVET	20.44±0.09

Data are mean ± SEM (n=3). BRPE: Pet ether extract of BR; BRCL: Chloroform extract of BR; BREA: Ethyl acetate extract of BR; BRET: Ethanol extract of BR; BVPE: Pet ether extract of BV; BVCL: Chloroform extract of BV; BVEA: Ethyl acetate extract of BV; BVET: Ethanol extract of BV

TABLE 2: TOTAL FLAVONOID MATTER IN DIVERSE EXTRACTS OF BR AND BV

Plant Extract	TFC (µg of Rutin equivalent/mg)
BRPE	15.33±0.13
BRCL	17.74±0.07
BREA	96.66±0.39
BRET	30.27±0.16
BVPE	15.22±0.75
BVCL	17.74±0.18
BVEA	106.67±0.42
BVET	28.82±0.63

Data are mean ± SEM (n=3). BRPE: Pet ether extract of BR; BRCL: Chloroform extract of BR; BREA: Ethyl acetate extract of BR; BRET: Ethanol extract of BR; BVPE: Pet ether extract of BV; BVCL: Chloroform extract of BV; BVEA: Ethyl acetate extract of BV; BVET: Ethanol extract of BV

Quantitative Scrutiny of Total Flavonoid Content (TFC): The flavonoid matter of BR and BV was established by $AlCl_3$ method. The extent of flavonoids in each extract was computed and stated as rutin equivalent/gm extract. It was quantitatively assessed and ranged from 15.33 to 96.66 RU/gm for different extracts of BR and 15.22 to 106.67RU/gm for various extracts of BV as specified in **Table 2.** Results exhibited that ethyl acetate extract of both plants covers highest extent of flavonoids with least content in pet ether extract. The TFC in BREA and BVEA was 96.66 of RU/gm and 106.67 of RU/gm of extract, respectively. Flavonoids are a group of polyphenols with tremendous radical foraging action and guarding organism from injury triggered by oxidative strain. Development of acid-stable composites with orthodihydroxy set in A or B ring of flavonoids & C4 keto set and also C3 or C5 hydroxyl set concerning flavanols & flavones configures the base of estimate. The foremost components for its bioactivity are amentoflavone, luteolin, cuppressoflavone, isovitexin, and isoorientin which may contribute to its wide pharmacological effects versus diverse ailments connected to oxidative strain [29].

***In-vitro* Antioxidant Assays:**

Estimation of DPPH Assay: *In-vitro* DPPH assay of different extracts of BV revealed the existence of antioxidant potential. The percent hindrance of DPPH radical scavenging activity is given in **Fig. 1.**

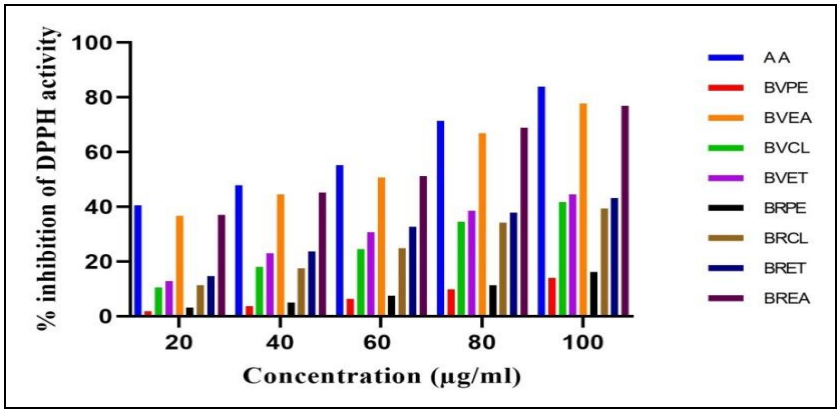

FIG. 1: THE PERCENT INHIBITION OF DPPH ASSAY OF DIFFERENT EXTRACTS OF BR, BV& ASCORBIC ACID. Data are mean±sem(n=3). Data were analysed by one way Anova followed by Dunnett's multiple comparison test. AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

The IC_{50} value for Ascorbic acid was attained to be 42.22µg/ml. The IC_{50} value was low for ethyl acetate extricate of BV and BR when compared to other extracts which was 49.81 and 48.95, respectively. The proximity of IC_{50} values of BREA and BVEA towards standard value proves

its high efficacy in foraging radicals **Fig. 2.** The results specify that plant extract was able to diminish the stable DPPH free radical to yellow-tinted diphenylpicryl hydrazine. The low IC_{50} of ethyl acetate extract gives an impression that BV and BR extracts contain specific active components that are competent of donating hydrogen to a free radical to eliminate odd electron that is liable for radical's reactivity. DPPH radical scavenging method has been verified to be beneficial as its outcomes are not altered by substrate polarity.

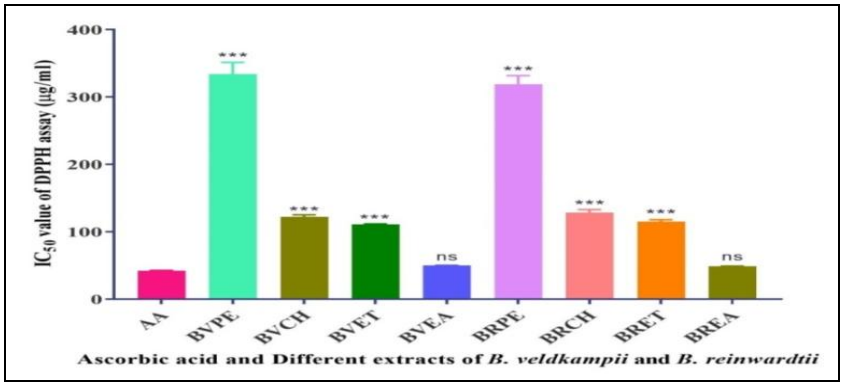

FIG. 2: THE IC50 VALUE OF DPPH ASSAY OF DIFFERENT EXTRACTS OF BR, BV& ASCORBIC ACID. Data are mean±sem (n=3). Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test. ***represents $p<0.001$ vs ascorbic acid, ns: not significant. AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

Estimation of Nitric Oxide Assay: The nitric oxide foraging capacity was concentration dependent with 100µg/ml scavenges most efficiently. The nitric oxide scavenging action of BR and BV is revealed in **Fig. 3** and correlated with known antioxidant Ascorbic acid. From the analysis of figures, we can conclude that scavenging effect was established to be good for ethyl acetate extract of both plants. The IC_{50} value **Fig. 4** of ascorbic acid, BVEA and BREA was found to be 52.32, 61.17 and 60.72, respectively. Both plants' percent hindrance and IC50 values demonstrate that both are exceptionally effectual in scavenging free radicals. In Nitric oxide assay, nitrite ions interact with Griess mixture, which turns out a purple azo dye. The extent of nitrites will be reduced in the existence of test constituents. The grade of decline in the evolution of purple azo dye will expose the level of hunting. Nitric oxide is produced by diverse cells like endothelial cells and macrophages. Nitric oxide reacts with oxidants and produces highly damaging peroxynitrite. Nitric oxide damage occurs via the peroxynitrite pathway because it can precisely oxidize LDL, resulting in irreversible harm to the cell sheath.

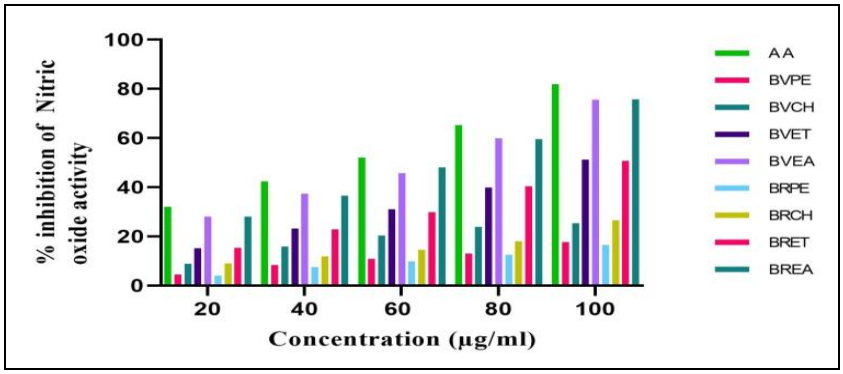

FIG. 3: THE PERCENTAGEINHIBITION OF NITRIC OXIDE SCAVENGING ASSAY OF DIFFERENT EXTRACTS OF BV, BR AND ASCORBIC ACID. Data are mean±sem (n=3). Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test.AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

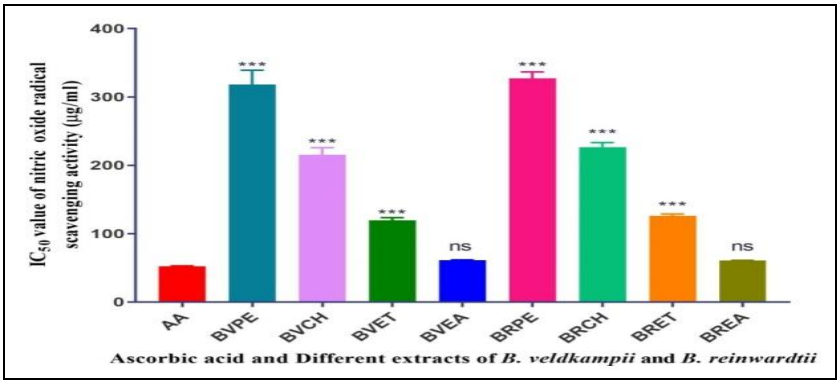

FIG. 4: THE IC_{50} VALUE OF NITRIC OXIDE SCAVENGING ASSAY OF DIFFERENT EXTRACTS OF BV, BR AND ASCORBIC ACID. Data are mean±sem (n=3). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. ***represents p<0.001 vs ascorbic acid, ns: not significant.AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

Estimation of ABTS Assay: The ABTS assay has been applied as an indicator that reveals the antioxidant activity of test samples. The scavenging activity of BV and BR tested at the concentration 20-100 µg/ml was assessed using ABTS cation assay and illustrated in **Fig. 5**.

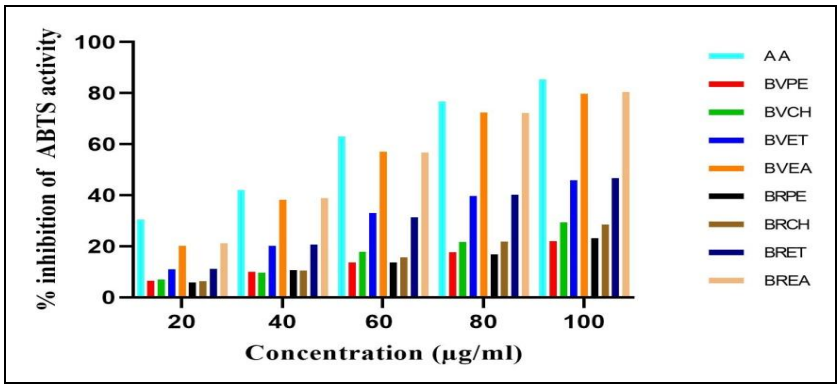

FIG. 5: THE PERCENTAGEINHIBITION OF ABTS RADICAL FORAGING ACTIVITY. Data are mean±sem (n=3). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

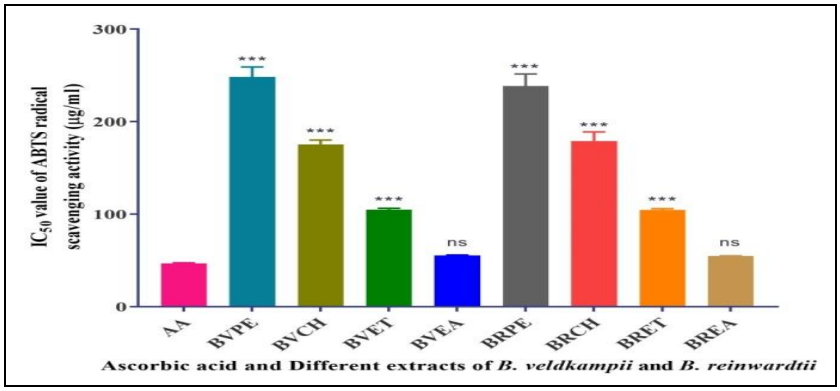

FIG. 6: THE IC_{50} VALUE OF ABTS ASSAY OF DIVERSE EXTRACTS OF BV, BR & ASCORBIC ACID. Data are mean±sem (n=3). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. ***represents p<0.001 vs ascorbic acid, ns: not significant.AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

Ethyl acetate extract of BV showed inhibitory percentage activity of 79.75 % at100 µg/ml. Ascorbic acid exhibited inhibitory activity versus ABTS cation from 30.44 to 85.46 % at

concentration ranges 20-100 µg/ml. The IC_{50} value of Ascorbic acid and BVEA was 46.8 and 55.45 µg/ml respectively **Fig. 6**. Similarly, percent hindrance of ethyl acetate extract of BREA ranges from 21.23to 80.49% at the concentration of 20 to 100µg/ml. BREA showed IC_{50} value of 54.85µg/ml. The percentage inhibition and IC_{50} values of BREA and BVEA proves that both are extremely efficacious in hunting free radicals. Abtscation radical is a blue chromophore made-up by the interaction between ABTS and potassium persulfate. Supplementation of the herbal extract to this pre-formed radical cationreduc edit to ABTS in a strength-dependent way.

Estimation of Superoxide Radical Scavenging Assay: Superoxide radical is considered a major biological source of ROS. Although it is a feeble oxidant, it furnishes the creation of potent and hazardous hydroxyl radicals and singlet oxygen, which cause oxidative strain together. The superoxide radical scavenging effect of obtains could culminate in the hindrance of OH radical formation. The percentage inhibition of Superoxide radical by pet ether and chloroform extracts of BV was instituted to be poor, as evident from **Fig. 7.** The ethyl acetate extract of BV exhibited considerable inhibitory activity at 74.27 % at its highest concentration used, and its IC_{50} value was 58.5µg/ml. From **Fig. 8**, it was noticed that the IC_{50} value of Ascorbic acid was 48.91 µg/ml.

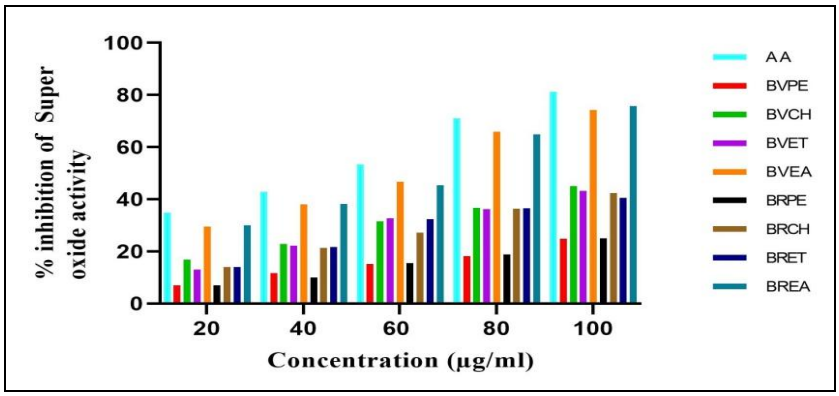

FIG. 7: THE PERCENTAGE INHIBITION OF SUPEROXIDE RADICAL SCAVENGING ACTIVITY. Data are mean±sem (n=3). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

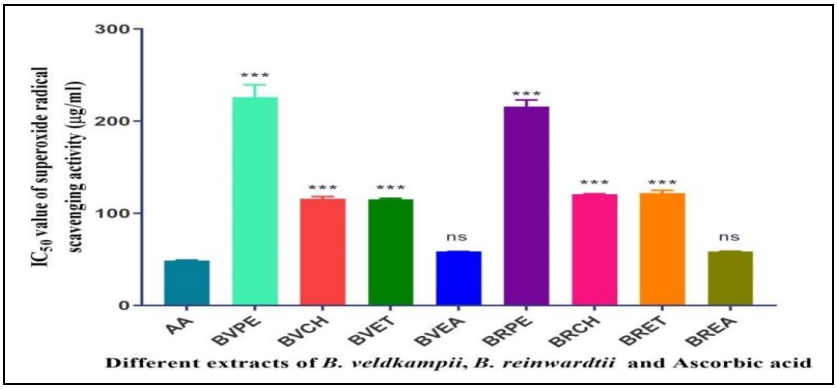

FIG. 8: THE IC_{50} VALUE OF SUPEROXIDE RADICAL HUNTING ASSAY OF ASCORBIC ACID AND DIVERSE EXTRACTS OF BV AND BR. Data are mean±sem(n=3). Data were analyzed by one way ANOVA followed by Dunnett's multiple comparison test. ***represents p<0.001 vs ascorbic acid, ns: not significant.AA: Ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET: BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

In case of various extracts of BR, remarkable effect was observed with ethyl acetate extract with inhibitory effect 75.73% at 100µg/ml and IC_{50} value 58.53µg/ml. The percentage hindrance of superoxide radical rummaging was established to be more in ethyl acetate extract of BV and BR and

was noted to be almost close to standard. The scavenging perspective will hinge on the number and positions of hydroxyl groupings in phenolic complexes in extracts.

Estimation of Hydrogen Peroxide Scavenging Assay: Ethyl acetate extract of BV and BR efficiently scavenged hydrogen peroxide that may be due to the occupancy of phenolic groupings that could contribute electrons to hydrogen peroxide, thus counteracting it into water. The results are depicted in **Fig. 9** and **Fig. 10**. Ascorbic acid was available to compare the scavenging power and ranged between 29.75 to 84.81% inhibition with IC_{50} value of 52.52µg/ml. The IC_{50} value of BVEA and BREA was 60.23 and 59.23 µg/ml, respectively. The percentage inhibition of Hydrogen peroxide scavenging action of BREA and BVEA was noted to be highly effectual as that of standard. Hydrogen peroxide occurs naturally at low intensity in the air, human body, plants, water, food, and microorganisms. Hydrogen peroxide is rapidly disintegrated into oxygen and water, producing OH radical, which initiates lipid peroxidation and triggers DNA damage.

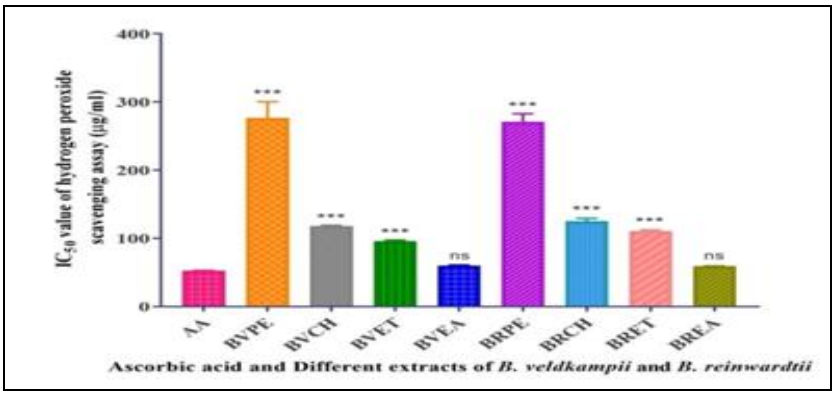

FIG. 9: THE PERCENTAGE INHIBITION OF HYDROGEN PEROXIDE RADICAL HUNTING ACTIVITY. Data are mean±sem (n=3). AA: ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET:BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

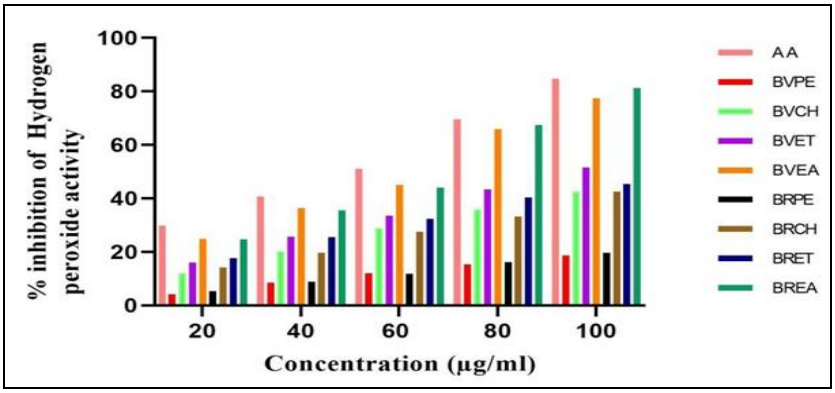

FIG. 10: THE IC_{50} VALUE OF HYDROGEN PEROXIDE SCAVENGING ASSAY OF ASCORBIC ACID AND DIVERSE EXTRACTS OF BV AND BR. Data are mean±sem (n=3). Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test.***represents p<0.001 vs ascorbic acid, ns: not significant. AA: ascorbic acid, BVPE: BV pet ether extract; BVCH: BV chloroform extract; BVET:BV ethanol extract; BVEA: BV ethyl acetate extract; BRPE: BR pet ether extract; BRCH: BR chloroform extract; BRET: BR ethanol extract; BREA: BR ethyl acetate extract.

CONCLUSION: Phytochemical examination of ethyl acetate extract of *B. reinwardtii* and *B. veldkampii* showed the occurrence of large amounts of flavonoids, essential oil, polysaccharides, phenolic and polyphenolic components. The main bioactive constituent present in these species is a biflavonoid compound, amentoflavone. It has been used traditionally in the treatment of various diseases. The *in-vitro* anti-oxidant investigations showed that these plant species possess strong *in-vitro* antioxidant and free radical hunting properties. Hence these plants used alone or in combination with other herbal drugs may exhibit

excellent antioxidant activities to protect the body from the deleterious effects of free radicals.

ACKNOWLEDGEMENT: The authors thank the management and staff of Ezhuthachan College of Pharmaceutical Sciences for constantly supporting and providing facilities during the research work.

CONFLICTS OF INTEREST: The author has no conflicts of interest in this work.

REFERENCE:

1. Jamshidi-Kia F, Wibowo JP, Elachouri M, Masumi R, Salehifard-Jouneghani A, Abolhasanzadeh Z and Lorigooini Z: Battle between plants as antioxidants with free radicals in human body. Journal of Herbmed Pharmacology 2020; 9(3): 191-9.
2. Nazir N, Zahoor M, Nisar M, Khan I, Ullah R and Alotaibi A: Antioxidants Isolated from *Elaeagnusum bellata* (Thunb.) Protect against Bacterial Infections and Diabetes in Streptozotocin-Induced Diabetic Rat Model. Molecules 2021; 26(15): 4464.
3. Li C, Hu Y, Nie Q, Chen S, Li G, Li L, Chen S, Tang B and Zhang J: A reactive oxygen species-responsive antioxidant nanotherapy for the treatment of drug-induced tissue and organ injury. Biomaterials Science 2020; 8(24): 7117-31.
4. Sanad FA, Ahmed SF and El-Tantawy WH: Antidiabetic and hypolipidemic potentials of *Solidago virgaurea* extract in alloxan-induced diabetes type 1. Archives of Physiology and Biochemistry 2022; 128(3): 716-23.
5. Checa J and Aran JM: Reactive oxygen species: drivers of physiological and pathological processes. Journal of Inflammation Research 2020; 13: 1057.
6. Sotoudeh R, Gholamnezhad Z and Aghaei A: The anti-diabetic and antioxidant effects of a combination of Commiphoramukul, Commiphoramyrrha and Terminaliachebula in diabetic rats. Avicenna Journal of Phytomedicine 2019; 9(5): 454.
7. Ma RH, Ni ZJ, Zhu YY, Thakur K, Zhang F, Zhang YY, Hu F, Zhang JG and Wei ZJ: A recent update on the multifaceted health benefits associated with ginger and its bioactive components. Food & Fun 2021; 12(2): 519-42.
8. Swallah MS, Sun H, Affoh R, Fu H and Yu H: Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. Int J of Food Sci 2020; 7: 2020.
9. Sreeshma LS and Nair BR: Induction of Apoptosis by Ethanolic Extracts of Two Species of Biophytum DC. (Oxalidaceae) in Four Human Cancer Cell Lines. International Journal of Pharmaceutical Sciences Review and Research 2015; 33(2): 149-53.
10. Sasidharan N: Biodiversity documentation for Kerala, Part 6: Flowering Plants. KFRI 6th edition 2004.
11. Varghese KJ, Anila J, Nagalekshmi R, Resiya S and Sonu J: Dasapushpam: The traditional uses and the therapeutic potential of ten sacred plants of Kerala state in India. International Journal of Pharmaceutical Sciences and Research 2010; 1(10): 50.
12. Sakthivel KM and Guruvayoorappan C: *Biophytum sensitivum*: Ancient medicine, modern targets. J of Advan Pharmaceutical Technology & Research 2012; 3(2): 83.
13. Jirovetz L, Buchbauer G, Wobus A, Shafi MP and Jose B: Medicinally used plants from India: Analysis of the essential oil of air-dried *Biophytum sensitivum* (L.) DC. Scientica Pharmaceutica 2004; 72(1): 87-96.
14. Sreeshma LS and Nair BR: Brine Shrimp Lethality Assay In Two Species of Biophytum Dc. (Oxalidaceae). International Journal of Pharmacy and Pharmaceutical Sciences 2014: 6(4); 582-6.
15. Anonymous: The Wealth of India (Raw materials), Vol. 2B.Publication and Information Directorate, Revised edition 1988.
16. Hajra PK, Nair VJ and Daniel P: Flora of India (Botanical survey of India, Calcutta) 1998.
17. Sadasivan SL and Nair BR: GC-MS analysis in two species of Biophytum DC. J of Pharma Res 2014; 8(4): 466-73.
18. Cooper JW and Gun C: Tutorial Pharmacy. CBS Publishers & Distributors Pvt. Ltd., Sixth Edition 1957.
19. Krithikar KR and Shah BD: Indian Medicinal Plants. Bio-Green Books, Second Edition 2012.
20. Singleton VL and Rossy JA: American Journal of Enology and Viticulture 1965; 16: 144.
21. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M and Trotin F: Phenolic compounds and antioxidant activities of Buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. Journal of Ethnopharmacology 2000; 72(1-2): 35-42.
22. Blois MS: Antioxidant Determinations by the Use of a Stable Free Radical. Nature 1958; 181: 1199-200.
23. Madan MP, Raghuvan G, Singh RAK and Palpo P: Free radical scavenging potention of Saussareacostus. Acta Pharmaceutica Sinica B2005; 55: 297-304.
24. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, and Rice-Evans C: Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Radical Biology and Medicine 1999; 26(9-10): 1231-7.
25. Nishikimi M, Rao NA and Yagi K: The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Res Communications 1972; 46(2): 849-54.
26. Ruch RJ, Cheng SJand Klaunig JE: Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechinsisolated from Chinese green tea. Carcinogenesis 1989; 10: 1003-8.
27. Rice-Evans CA, Miller NJ and Paganga G: Antioxidant Properties of Phenolic Compounds. Trends in Plant Science 1997; 2: 152-9.
28. Pietta PG: Flavonoids as Antioxidants. Journal of Natural Products 2000: 63(7); 1035-42.
29. Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini AG and Bogdanov S: Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 2004; 15(4): 235-40.

How to cite this article:
Nair C, Himacs and Asheeta A: *In-vitro* antioxidant and free radical scavenging activity of whole plant extract of *Biophytum* species. Int J Pharm Sci & Res 2023; 14(4): 1828-37. doi: 10.13040/IJPSR.0975-8232.14(4).1828-37.