Research Article

Ethnobotanical Validation: Antioxidant and Analgesic Activities of Some Species of Genus Brachystelma

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ABSTRACT

Introduction: Brachystelma is a small subterranean tuber belonging to the Asclepiadaceae family. The leaves and tubers are nutrient-dense and delicious, and are used in traditional medicine to treat pain.

Aim: *Brachystelma malwanense*, *Brachystelma naorojii*, and *Brachystelma edule* were the three species chosen; however, no experimental studies have been conducted to support this traditional use. The primary goal of this study was to investigate the analgesic and antioxidant properties of the tubers.

Methods: The acetic acid-induced writhing method was used to test the analgesic activity in mice. Antioxidant potential was assessed using a range of methods, such as 1,1-diphenyl-2 pricrylhydrazyl, FRAP, Reducing power assay, polyphenols, carotenoids, and ascorbic acid.

Findings: The aqueous extract of tubers of B. naorojii at the 500 mg/kg dose level exhibited significant activity compared with the other tubers. No behavioral abnormality or mortality was observed in rats treated with crude tubers in any of selected plants. In antioxidant evaluation, ascorbic acid (80.92 ± 0.000 mg/100 g), DPPH scavenging activity (88.32 ± 0.56% inhibition), superoxide dismutase (0.004 ± 0.000 ΔOD min. -1 mg-1) and the total antioxidant activity (0.650 ± 0.001 mg AAE/100 g) was higher in the tubers of B. malwanense than in the others. The Polyphenols (52.6 ± 1.039 mg/100 g), FRAP (0.492 ± 0.014 mg AAE/100 g), peroxidase (0.013± 0.000 ΔOD min.) activities were higher in the B. naorojii tubers. The notable antioxidant properties exhibited by Brachystelma tubers may be ascribed to their polyphenol concentration and additional phytochemical components.

Conclusion: The results of this study validated the traditional use of the plant for pain relief by indicating that the tuber extract has potent analgesic and antioxidant properties, which may be related to its polyphenol concentration.

Keywords: Analgesic, antioxidant, Ethnobotany, Brachystelma tubers.

INTRODUCTION

Robert Brown initially described Brachystelma in 1822. The Brachystelma R. Br. genus, which belongs to the Asclepiadaceae family, comprises approximately 100-120 species [1,2,3] globally. These species are primarily found in Southeast Asia, South Africa, and Australia [4]. As of now, 22 species have been identified in India, with the majority occurring in the Peninsular region. Among these, 21 are endemic [5], and 3 are present in Maharashtra. Brachystelma, a genus within the Asclepiadaceae family, is derived from the Greek etymological roots. The term combines "Brachy," signifying short, and "stelma," denoting crown. These plant species inhabit environments characterized by partially degraded hillsides and exposed summits that coexist with gramineous vegetation. Brachystelma is taxonomically classified as a diminutive perennial herb that exhibits tuberous root structures. Its natural distribution encompasses eroded slopes and denuded hilltops that are interspersed with graminoid vegetation. In rural regions, individuals employ tubers of these plants as traditional remedies for various ailments, including cephalalgia, gastrointestinal discomfort, and pediatric upper respiratory infections. The tubers of *B. edule* are considered edible in China, where the plant is utilized to treat coughs and reduce mucus production. In India, approximately 800 wild edible plant species are consumed by the tribal people [6]. Western Maharashtra constituted the primary focus of the majority of surveys conducted. In a study of mountainous regions across Western Maharashtra and Goa, Vartak [7] identified 120 species of flowering plants and ferns utilized as food sources by indigenous communities. Among these, 58 species were consumed regularly, 27 were utilized infrequently, and 35 relied exclusively on periods of severe food scarcity. Phytochemicals that are used to manufacture various bioactive molecules in a precise and selective manner are found in medicinal plants. Both primary and secondary metabolites are produced by plants [8]. The fact that many Brachystelma species have different names across various ethnic groups is a sign of their significance. Tubers are used more frequently than other plant components, such as leaves, roots, and stems. Given their widespread use in numerous nations, this suggests that tubers are highly prioritized in ethnobotany [9]. Numerous species of Brachystelma are characterized by their capacity to accumulate substantial quantities of water and nutrients in their subterranean structures, particularly their tubers.

This attribute renders them exceptionally valuable food resources. Brachystelma tubers are harvested and consumed by certain wild animals. Based on casual observations, Brachystelma species have been investigated for their nutritional content in various animals [10]. Plants have the ability to produce secondary metabolites, which can be utilized in the identification and creation of new medicinal compounds [11]. Extracts from medicinal plants may be used to develop novel drugs that are effective against diseases that are difficult to cure. An uncommon medicinal plant in the Euphorbiaceae family is *Euphorbia fusiformis*. While tuber latex is used to treat chronic wounds, skin conditions, liver problems, and diarrhoea, the dried root powder and fresh rhizome of *E. fusiformis* are used to boost the mother's milk secretion. Tubers are abundant in a range of primary and secondary metabolites, including carbohydrates, alkaloids, vitamins C and E, flavonoids, phenols, glycosides, saponin, and minerals, according to phytochemical screening. According to current research, the contents of microelements provide tubers with strong antioxidant properties.[12]

MATERIALS AND METHODS

Collection of Plant Material: Plant material was collected during the rainy season (June-September) and summer (May–June) from various locations in the districts of Satara, Kolhapur, Pune, and Sindhudurg. The specimens comprised leaves and tubers from three Brachystelma species: *B. edule* Collett and Helmsl., *B. naorojii* P. Tetali, D. K. Kulk., S. Tetali & M. S. Kumbhojkar, and *B. malwanense* S. R. Yadav & N. P. Singh. Additionally, potato tubers were obtained. The plants are in a vegetative state during the rainy season and exhibit flowering and fruiting characteristics in the summer [13]. The plant species were verified and authenticated using references from the 'Flora of Maharashtra' [14] and 'Flora of Kolhapur District' [15]. To mitigate moisture loss during transportation to the laboratory, the collected plant samples were placed in a polyethylene bag.

Sample Preparation: The wild edible tubers freshly harvested and devoid of aerial components, underwent a thorough cleaning process utilizing water to eliminate soil particles and extraneous matter, which were then blotted until the loss of excessive moisture and weight gained fresh weight. Afterwards, the tubers were chopped and deposited in a paper envelop (blotting paper) and kept in an oven at 60°C for drying until a static weight was obtained. The completely dried specimen was ground into a fine powder using an electric grinding device. Powdered samples were subsequently transferred to sealed plastic containers for preservation and nutrient analysis. All analytical procedures were repeated three times using reagents of analytical grade quality.

Non enzymatic antioxidant analysis

Carotenoids: Carotenoids were enumerated according to the method described in [16].

Total polyphenol: Total polyphenol content in plant material was determined according to a previously described method [17]. Using the acetone extract prepared for chlorophyll analysis, 2 ml of plant extract was transferred to Nessler tubes. This sample was combined with 10 ml of 20% Na_2CO_3 solution. The mixture was then diluted with distilled water to achieve a total volume of 35 ml. To this blended included 2 ml of Folin-Denis reagent, blended completely diluted up to final volume 50 ml upon distilled water. For the readiness of standard polyphenols bend tannic acid solution 0.1 mg ml-1 was used, and a blank was set up except for polyphenol. A UV-VIS double-beam spectrophotometer was used to measure the absorbance at 660 nm after the development of color.

Ascorbic Acid (Vitamin-C): The ascorbic acid substance in the plant material was evaluated agreeing the techniques of Sadasivam and Manikam [18] is a titrimetric method. A 500 mg sample of fresh plant material was combined with 10 ml of 4% oxalic acid in a mortar and pestle. This procedure aims to reduce the pH and stabilize the contents by initiating the catalytic oxidation. The resulting mixture was subsequently filtered using a Buchner funnel with Whatman No. 1 filter paper. The filtrate was collected in a conical flask and subsequently used to determine the ascorbic acid content.

Free radical scavenging activity Dry powder of the plant material was extracted in methanol using an orbital shaker for approximately four to five hours and subsequently filtered with Whatman No. 1 filter paper. The filtrate was transferred to an evaporating dish and concentrated to a small volume in a water bath before being allowed to dry. The concentrates were weighed, and the yield values were determined.

DPPH radical scavenging activity: The method described by Wang et al. [19] was employed to evaluate DPPH radical scavenging activity. To assess the free radical-scavenging capacity of the extracts, the investigators utilized a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) radical was used. Fifteen hundred µl of each plant extract were included, at an equivalent volume, to methanolic solution of DPPH (100 uM) were mixed. The solution blends were placed in the dark for 30 min, and the optical density was measured at 517 nm using a double-beam spectrophotometer. The standard was prepared using ascorbic acid at a concentration of 10 mg/ml, and methanol functioned as the blank in the experiment.

Ferric Reducing Antioxidant Power (FRAP): Cancer prevention agent movement measures were trailed by the technique for Benzie and Strain [20]. The total antioxidant capacity of the plant extracts was evaluated using the ferric reducing antioxidant power (FRAP) assay. A mixture of plant extract (0.5 ml plant extract and an equivalent volume of distilled water was combined with 3 ml of FRAP reagent, homogenized, and subsequently incubated at 37°C for 15 min. A control sample was prepared without plant extract. The absorbance of the resultant blue-colored complex was measured spectrophotometrically at 593 nm. The results were expressed as ascorbic acid equivalent antioxidant capacity.

Reducing power Assay: The reducing capacity of the methanolic extract was evaluated using the Oyaizu technique [21]. A test tube containing 0.5 ml of plant extract (ranging from 0.2 to 1 mg/ml) was combined with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% potassium ferrocyanide [K_3Fe (CN) $_6$]. This mixture was agitated and maintained at 50°C for 20 min. Following incubation, 1 ml of 1% TCA was added to terminate the reaction and the solution was centrifuged at 3000 rpm for 10 min. The top 1.5 ml of the resulting liquid was mixed with an equal amount of distilled water and 0.1% $FeCl_3$ solution (0.1 mL). After a 10-minute incubation period, the absorbance was determined at 700 nm using a spectrophotometer. Ascorbic acid was used as a standard (100 µg/ml). The absorbance of the response blend was higher, indicating a higher reducing power.

Ferrous ion-chelating ability assay: The ferrous ion-chelating capacity of the plant extract was evaluated using a modified approach based on the Decker and Welch's method [22]. In this procedure, 0.1 ml the methanolic plant extract was introduced into a test tube along with 0.1 ml of 2 mM $FeCl_2$. The reaction was initiated upon the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was homogenized and incubated for approximately 10 min at ambient temperature. Subsequently, the absorbance was measured using a spectrophotometer at 562 nm. A blank sample was prepared by substituting distilled water for the ferrozine solution, whereas the control used distilled water instead of the plant extract. Ascorbic acid was used as a reference standard. All estimations were performed in triplicate.

Total antioxidant capacity (TAC) by the phosphomolybdenum method: The method described by Prieto et al. [23] was employed to evaluate the total antioxidant capacity of the methanolic plant concentrate. The procedure involved combining the methanolic plant extract (0.5 mL) with 5 ml of the phosphomolybdenum reagent in a test tube. This reagent consisted of 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid dissolved in 250 ml of distilled water. The tubes were then sealed and placed in a water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the mixture was measured using a Spectronic 20 visible spectrophotometer at 695 nm. A blank was prepared using 5 ml of the reagent solution and an equivalent volume of methanol and subjected to the same incubation conditions. L-Ascorbic acid was used as the standard for this assay.

Enzymatic antioxidant:

Catalase: Catalase activity was assessed following the protocol outlined by Sadasivam and Manikam, which was adapted from Luck's method [24]. Freshly harvested tubers and leaves underwent washing, drying, and dicing. A 0.5 g portion of the diced material was homogenized in 10 ml of ice-cold phosphate buffer (1/15M, pH 6.8) and filtered through four layers of muslin. The filtrate was centrifuged at 10,000 rpm for 20 min at 4°C. The resulting supernatant was used as an enzyme source. The reaction mixture comprised 3 ml of 0.05% H_2O_2 in 100 ml phosphate buffer (pH 7) and 0.1 ml of the enzyme extract. Upon thorough mixing, the change in the optical density per minute at 240 nm was promptly measured using a Shimadzu-190 UV-VIS double-beam spectrophotometer. The soluble protein content of the enzyme extract was determined using the method described by Lowry et al. [25]. Enzyme activity was expressed in units of min-1 mg-1 protein, according to Bergmeyer's [26] guidelines.

Peroxidase: Peroxidase enzyme analysis was performed using Maehly's technique [27]. Plant material (500 mg, fresh) from leaves and tubers was separately homogenized in 15 ml of ice-cold phosphate buffer (0.1 M, pH-7) and filtered through a four-layered muslin cloth. The filtrate was centrifuged at 10,000 rpm for 20 min and the resulting supernatant served as the enzyme source. A reaction mixture was prepared, consisting of 2 ml phosphate buffer (0.1M, pH-7), 1 ml guaiacol (20 mM), and 1 ml enzyme extract. The reaction commenced upon the addition of 0.05 ml H_2O_2 (1 mM). The optical density changes resulting from guaiacol oxidation were recorded after 30 min at 470 nm. The enzyme activity was quantified in minutes. -1 mg. -1 Protein.

Superoxide dismutase: Superoxide dismutase activity was assessed utilizing a modified version of the technique delineated by Giannopolitis and Ries [28]. The enzyme extraction process involved homogenizing 500 mg of fresh plant material in 15 ml of chilled 150 mM potassium phosphate buffer (pH 7.8) with 1% PVP to protect the enzyme from polyphenolic compounds. The homogenate was filtered through four layers of muslin cloth and the filtrate was subsequently centrifuged at 10,000 rpm for 20 minutes at 0 to 4°C. The resultant supernatant served as the enzyme source. The assay mixture consisted of 2 ml potassium phosphate buffer (pH 7.8), 0.2 ml methionine (13 mM), 0.1 ml Nitroblue tetrazolium (75 µM), 0.5 ml EDTA (0.1 mM), 0.1 ml enzyme, and 0.1 ml riboflavin (2 M) added last. The final volume was adjusted to 3 ml, and the absorbance was immediately recorded at 560 nm using a UV-VIS double-beam spectrophotometer (Shimadzu-190, Japan). The mixture was subsequently exposed to full daylight for 30 min, after which the absorbance was measured at 560 nm. The enzyme activity was expressed as ΔOD min.-1 mg. -1 of protein.

Toxicity and Safety Evaluation of plants:

Acute toxicity study: [29,30] Potion was opted by applying acute toxicity review (OECD, 423). A study was conducted to evaluate the acute toxicity of methanolic extracts derived from the tubers of *B. edule, B. naorojii*, and *B. malwanense* in rats. The test subjects fasted overnight and were kept under standard conditions before the experiment. To assess the LD50 of the natural tuber crude drug, four groups of six rats each were orally administered PM at doses ranging from 500 to 5000 mg/kg. The animals were monitored for behavioral changes and mortality at regular intervals: every 5 min for the initial 30 min, and then at 2, 4, 8, and 24 h

post-treatment. Observations were continued for a week to track any deaths. The results showed that no fatalities occurred within 7 days following the administration of the crude tuber drug from all three species, suggesting that doses up to 5000 mg/kg were considered safe.

Dose selection: [31] Dosage was chosen based on acute oral lethality analysis performed on crude tuber drug. The study revealed protection at dosages of up to 5000 mg/kg, with no observed behavioral irregularities or fatalities within 48 h post-administration. Acute toxicity indicators were not detected. As a result, researchers established the median dose for rat trials by selecting one-tenth of the 5000 mg/kg dose, equating to 500 mg/kg of the crude tuber drug. This 500 mg/kg dosage was subsequently chosen for the rat experiments.

Analgesic Activity: Acetic acid-induced writhing in mice: [32,33]. The acetic acid-induced writhing strategy was embraced for assessment of pain-relieving action. The characteristic signs of writhing include persistent sideways pressure, elongation of the rear legs, and abdominal muscle contractions that bring the mouse's intestines into contact with the ground. This behavior is also marked by the twisting motion of the upper body. Any instance of writhing was regarded as a positive indicator. Swiss albino mice weighing between 15 to 35 g were used for the assessment of pain-relieving movement; in each group, six albino mice were kept. Acetic acid solution (1% v/v) was added to distilled water. A solution of aspirin (Acetyl Salicylic acid; dose-100 mg/kg) was prepared in typical saline water. Various suspensions of *Brachystelma edule, B. naorojii* and *B. malwanense* (dose-500 mg/kg each) in distilled water were prepared as test (drug) solutions. Five separate groups of Wistar albino mice were established, each containing six animals that were examined individually. The animals were deprived of food for 12 hours before drug administration until the experiment's completion. After weighing, the mice were appropriately numbered. Both standard and test medications were administered orally. One hour later, writhing was induced by injecting 1% acetic acid intraperitoneally at a volume of 0.1 ml per 10 g of body weight. Writhing episodes were observed and recorded for 20 min; these included arching of the back, body elongation, and hind limb extension, which were counted as stretching movements.

RESULTS

The goal of this study was to assess the traditional claims for the aforementioned uses of Brachystelma species from a scientific perspective.

Non enzymatic antioxidant:

Antioxidants are found in many foods including tubers, fruits, and vegetables. These are also available as dietary supplements. Antioxidants are natural substances that may prevent or delay cell damage.

The Polyphenol, ascorbic acid, and carotenoid contents in the tubers of *B. edule, B. naorojii, and B. malwanense* are shown in (Fig. 1). Polyphenols were higher in the *B. naorojii* tuber (52.6 ± 1.039 mg/100 g fresh weight) and lower in *B. edule* (45.7 ± 2.651 mg/100 g fresh weight). Ascorbic acid was higher in *B. malwanense*, (80.92 ± 0.000 mg/100 g Fresh weight) followed by *B. naorojii and B. edule* (50.42 ± 1.525 mg/100, 46.24 ± 2.831 mg/100, Fresh weight respectively). Overall, carotenoid content was lower in all three tubers than in the other antioxidants. The highest carotenoid content found in tubers of *B. edule* was 0.73 ± 0.057 mg/100 g fresh weight. Carotenoid functions protective against damage by oxygen and light. Among them, tubers of *B. edule* were rich in carotenoids, *those of B. naorojii* were rich in polyphenols, and *those of B. malwanense* were rich in ascorbic acid. According to the Food and Nutrition Board, the RDA for Vitamin-C intake is 60 mg/day. In the present study wild edible tubers of *B. malwanense* meet the everyday aid standard for Vitamin-C and tubers of *B. edule and B. naorojii* was slightly lower values than the RDA [34].

Free radical scavenging activity

The results of the free radical scavenging activities, including DPPH and ferrous ion chelation, are presented in (Fig. 2). Among the species investigated, *B. malwanense* tubers demonstrated the highest DPPH scavenging activity with 88.32 ± 0.56% inhibition. In contrast, B. naorojii exhibited the lowest DPPH scavenging activity, attaining 78.72 ± 0.18% inhibition. Whereas ferrous ion chelating activity found to be higher in the methanolic extract of *B. edule* tubers (58.25 ± 0.54% inhibition) followed by the *B. naorojii* (55.50 ± 1.03% inhibition) and *B. malwanense* (48.90 ± 2.34% inhibition). DPPH radical scavenging activity is a sensitive system for antioxidant screening of plant extracts [35].

The FRAP and **total antioxidant** activities of the tubers of Brachystelma species are depicted in the (Fig. 3). FRAP activity was high in tubers of *B. naorojii* (0.492 ± 0.014 mg AAE/100 g dry weight) and low in tubers of B. *edule* (0.372 ± 0.006 mg AAE/100 g dry weight). A higher FRAP value indicated the bioactive potential of the plant. The total antioxidant activity of tubers of *B. malwanense* (0.650 ± 0.001 mg AAE/100 g dry weight) was higher than that of tubers of *B. naorojii* (0.585 ± 0.000 mg AAE/100 g dry weight).

The reducing power (Fig. 4) of the methanolic extracts of the tubers of *B. malwanense* (0.482 ± 0.027 relative absorbance) was higher than those of the tubers of *B. edule* (0.436 ± 0.001 relative absorbance) and *B. naorojii* (0.385 ± 0.000 relative absorbance). The free radical-scavenging properties of antioxidants found in medicinal plants play a crucial role in preventing diseases and providing therapeutic benefits.

Enzymatic Antioxidants: Enzymatic antioxidants such as peroxidase, catalase, and superoxide dismutase were studied in the tubers of Brachystelma species, and the results are depicted in the (Fig. 5). Among the studied plants, *B. naorojii* tubers exhibited the highest peroxidase

activity (0.013 ± 0.000 ΔOD min. -1 mg-1 protein), while *B. malwanense* showed the greatest catalase activity (0.162 ± 0.002 ΔOD min. -1 mg-1 protein). Superoxide dismutase levels were the highest in *B. malwanense* (0.004 ± 0.000 ΔOD min. -1 mg-1 protein) and lowest in *B. naorojii* (0.002 ± 0.000 ΔOD min. -1mg-1 protein). In food, peroxidase enzymes can affect not only taste, bitterness, astringency, and color, but also interact with proteins, potentially reducing digestibility and desirability, thus diminishing the nutritional value of foods. The author (36) measured superoxide dismutase activity in *Ceropegia bulbosa* tubers (0.30 ± 0.005-unit min. -1 mg-1 protein) and *B. edule* (0.21 ± 0.004-unit min. -1 mg-1 protein). The current study found lower superoxide activity in Brachystelma species than in previous studies, likely because of differences in climatic conditions.

The current investigation sought to examine the in vitro antioxidant efficacy of methanolic extracts obtained from tubers of *B. edule, B. naorojii*, and *B. malwanense*. Based on the experimental outcomes, the Brachystelma plant demonstrated significant antioxidant potential. This information could be beneficial for further work carried out to isolate these compounds and screen for their biological activities.

Analgesic activity Swiss albino mice were used to examine analgesic efficacy using the acetic acid-induced writhing method, with aspirin (100 mg/kg body weight) serving as the standard for comparison. The study revealed that the aqueous extract of *B. naorojii* tuber exhibited significant pain relieving effects at 500 mg/kg body weight, comparable to that of the standard Aspirin (Fig.-6). Assessment of analgesic properties showed a significant ($P<0.001$) decrease in paw plunging response for *B. edule, B. naorojii*, and *B. malwanense* (all at 500 mg/kg) compared to the control group. These observations indicate that the aqueous extracts of *B. edule, B. naorojii*, and *B. malwanense* potentially possess analgesic properties. Everyone these impacts and the alterations in the demeanor activities may be advised as conductive results to the utilization of *B. naorojii* tuber in the administration of painful and inflammatory conditions. Preliminary phytochemical screening indicated the presence of terpenoids, volatile oils, tannins, flavonoids, and glycosides separated from the plant extracts to produce analgesic and anti-inflammatory effects. In recent medicines huge of triterpenoid glycosides are used in analgesic and anti-inflammatory agents.

The mice exhibited various behavioral changes, including stretching, leaning to one side, extending their hind legs, contracting their abdomens, touching the floor, and twisting their trunks, which were considered positive writhing responses (Plate-4). Both test and reference drugs significantly ($P<0.001$) decreased the number of abdominal constrictions and hind leg stretches induced by acetic acid injections in a dose-dependent manner. All test and standard drug groups demonstrated a significant ($P<0.001$) reduction in pain compared to the control group. Among the two species studied, the pain-relieving effect of the B. naorojii tuber most closely resembled that of the standard drug aspirin (Fig. 6). This proved that the tuber of *B. naorojii* (Plate -2) had significant analgesic activity and the least activity was found in *B. edule* (Plate 1) and *B. malwanense* (Plate 3). No behavioral abnormality or mortality was observed in the rats treated with crude tubers of *B. edule, B. naorojii, and B. malwanense;* hence, these tubers are safe for consumption. Tubers of *B. naorojii* exhibit significant analgesic activity. It was decided that the *B. edule, B. naorojii*, and *B. malwanense* aqueous extracts obtained analgesic characteristics, which are eventual mechanisms. The extract will, therefore come of promising advantage in the disposition of inflammatory disorders and pain.

These findings suggest that the pain-relieving effects of the extract may involve both central and peripheral mechanisms of action. Subsequent studies should investigate the contributions of triterpenoids, flavonoids, and alkaloids. Numerous researchers have conducted comparable investigations of the pain-relieving and antioxidant qualities of various plant species.

Plate 1: *Brachystelma edule* Collett and Helmsl

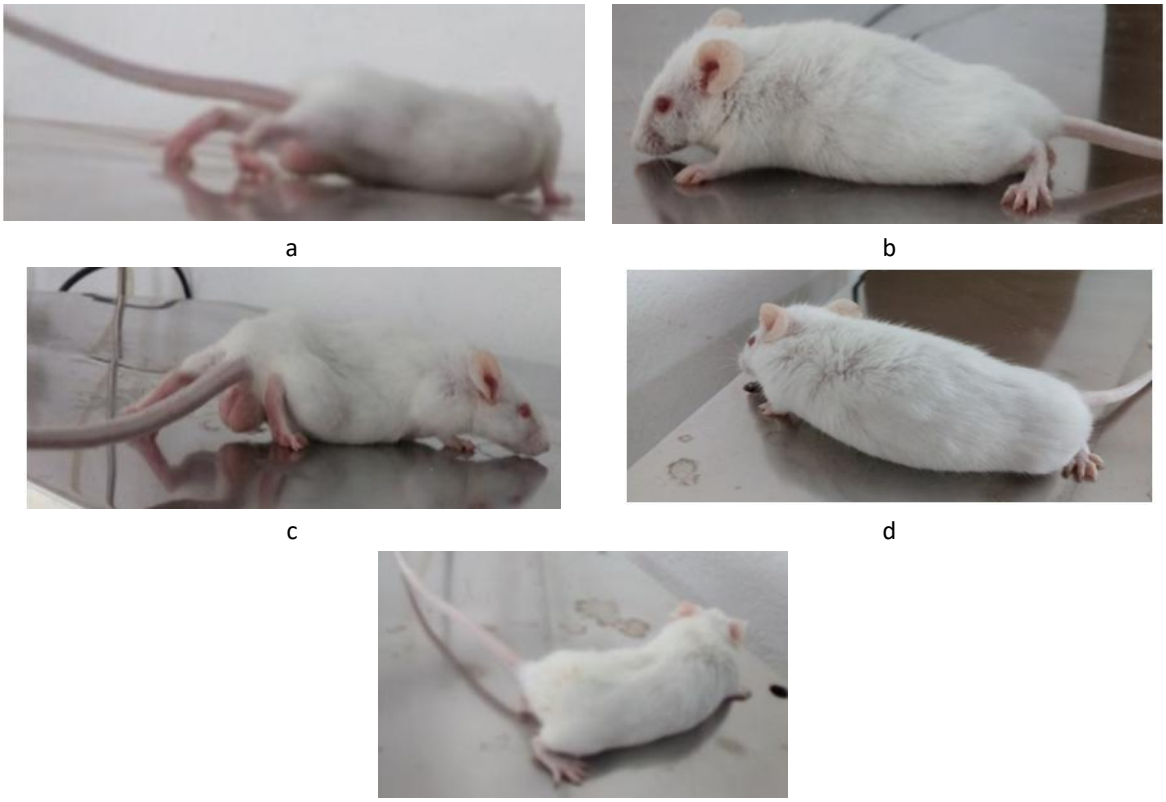
Plate 2: *Brachystelma naorojii* P. Tetali, D. K. Kulk., S. Tetali & M. S. Kumbhojkar

Plate 3: *Brachystelma malwanense* S. R. Yadav and N. P. Singh

DISCUSSION

Many diseases, including heart diseases and some types of cancer, may be prevented by eating a diet rich in antioxidants. Antioxidants prevent or reduce oxidative damage by scavenging free radicals in cells. The research on the beneficial effects of antioxidants is ongoing worldwide. Tuber extracts of selected Brachystelma species have long been used in India as an analgesic, nutritional and anti-inflammatory medication; however, no proof of the plant's analgesic, anti-inflammatory and antioxidant efficacy in pain and inflammation models has been discovered [37,38,39,40]. In Ethiopian folk medicine, traditional healers commonly utilize *Echinops kebericho* (Asteraceae) to relieve pain and inflammation. However, the plant's traditionally claimed usage has not been properly assessed. The results of this study showed that the extract possessed significant analgesic and anti-inflammatory activities [41]. Chloroform extract exhibited notable sedative, analgesic, and anti-inflammatory properties. This study provides compelling evidence for the traditional use of *Diospyros kaki* to alleviate pain, inflammation, and sleeplessness [42]. The aqueous extract of *Murya koenigii*, administered orally at 500 mg/kg, significantly reduced carrageenan-induced edema throughout all stages of the study. The hot-plate method was used for present imvestigation, an aqueous extract of *M. koenigii* Linn. substantially and dose-dependently reduced the number of acetic acid-induced writhings, notably increasing the time before paw licking [43]. Research on the analgesic and antioxidant capabilities of *Petersianthus macrocarpus* extract and solvent fractions revealed that the extract's analgesic and antioxidant qualities are primarily found in the polar fractions. The extract caused a significant ($P < 0.05$) increase in pain threshold on the hotplate at 1000 mg/kg, but an inconsequential ($P > 0.05$) increase at 200 and 500 mg/kg. The extract significantly reduced ($P < 0.05$) acetic acid-induced writhing in mice in a dose-dependent manner. Fractionation enhanced the analgesic effects of the ethyl acetate and aqueous fractions substantially ($P < 0.05$) at 200 mg/kg. The extract demonstrated weak iron chelating abilities, strong reducing power, and high DPPH radical scavenging activity, with an IC of 0.05 mg/ml. The total phenol content was measured at 142.32 mg in terms of gallic acid. The ethyl acetate and aqueous fractions exhibit more potent antioxidant effects [44]. Research has shown that the aqueous portion of *Haloxylon salicornicum* exhibits various beneficial properties, including anti-inflammatory, fever-reducing, pain-relieving, and antioxidant effects without significant toxic side effects. Consequently, HEW is considered safe for medicinal use and may be beneficial for treating diverse health conditions [45]. A comprehensive evaluation of the traditional uses, nutritional content, chemical composition, and biological effects of Brachystelma species has been conducted 46. Although many Brachystelma species are traditionally consumed as wild foods, only *B. edule* and *B. naorojii* have been scientifically analyzed for their nutritional value. Assessing the nutritional content of wild edible plants is crucial to support their inclusion in the diet[47-50]. Research by the author [51] indicates that *B.edule* tubers could serve as a valuable source of nutrients, such as proteins, fibers, and carbohydrates. Within the Ceropegieae tribe, Brachystelma has received limited attention for the measurement of its phytochemical components. Various plants have been utilized in traditional medicine to treat dental pain, strengthen gums, expel parasites, address kidney issues, alleviate pain, reduce inflammation, protect the liver, lower blood sugar levels, and combat cancer. The ethanolic extract of *Toona ciliata* heartwood demonstrated significant pain-relieving properties compared with other extracts, as confirmed by the tail immersion method [52]. This study explored the analgesic qualities of fruit extracts from *Sida tiagii* Bhandari. The extraction process involved grinding the fruits with 90% ethanol, followed by n-hexane (HS) and ethyl acetate (EAS) extraction. Residual ethanol extract (RES) was also produced by water bath drying. Swiss albino mice were administered oral doses of 200 and 500 mg/kg body weight of each extract. The pain-relieving effects were assessed using acetic acid-induced writhing, tail immersion, and tail-flick tests. The findings revealed that the ethanolic extract of *S. tiagii* exhibited potent analgesic properties, whereas the EAS extract demonstrated pain alleviating effects [53]. In Africa, plants have traditionally been used as a rich source of medicinal compounds. In developing nations, the prevalence of obesity is sharply rising. It is conceivable that anorectic plants reduce excessive weight gain. Finding the phenolic compound content of five Burkina Faso anorectic potential plants, *B. bingeri*, was one of them. This study showed that anorectic plant extracts have a strong antioxidant capability, which is essential for any weight-loss activity. They can also inhibit the enzyme acetylcholinesterase [54]. One way to address the growing demand for organic healthcare goods is through the use of herbal extracts. The medicinal properties of *Haplophyllum tuberculatum* (Forsskal) A. Juss (*H. tuberculatum*) extracts have been used to treat diverse ailments. This investigation seeks to examine the antioxidant, pain-relieving, anti-inflammatory, and wound-healing efficacy of both water-based and ethanol-based extracts obtained from this plant [55]. Experimental results indicated that the ethanol-based extract demonstrated exceptional performance in antioxidant assays (DPPH, FRAP, and TAC). Conversely, the water-based extract exhibited remarkable effectiveness in live animal studies, showing substantial pain-relieving, anti-inflammatory, and healing potentials. By reducing pain and inflammation, and accelerating wound healing, these results lend credence to the plant's potential for medicinal use. Further research on this species is required to identify and isolate chemicals that may have therapeutic and medical applications. *Pueraria tuberosa* Linn. (PT) is a perennial climber belonging to the Leguminosae family that grows throughout India's tropical regions. It is the preferred medication in the Ayurvedic medical system for treating pain, inflammation, and other associated conditions. *P. tuberosa's* methanolic and hexane extract fractions have strong antioxidant qualities. The antioxidant effects of

polyphenols are mostly due to glycosidic substitution, which lowers the number of free hydroxyl groups in the compounds[56]. This study aimed to examine the pain-relieving and antioxidant capabilities of three medicinal plants originating from Bangladesh [57]. In mice, the three plant extracts have decreased pain in a dose-dependent way using both of these techniques. These three plant species have been used traditionally as analgesics and for wound healing, among other purposes.



Plant 4: Analgesic activity Mice showed stretch, tension to one side, extension of hind legs (a, c), and contraction of abdomen touches the Flore (b, d), turning of trunk (twist) (e) since writhing as a positive response.

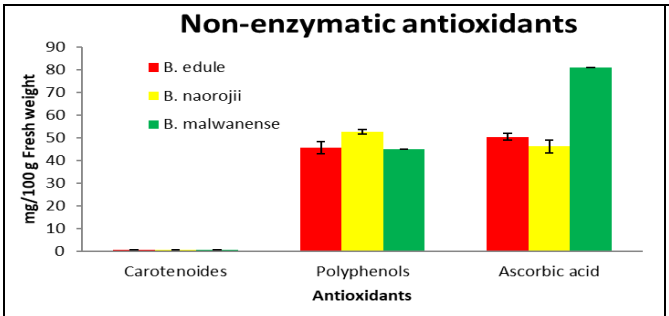


Figure 1: Non-enzymatic antioxidants

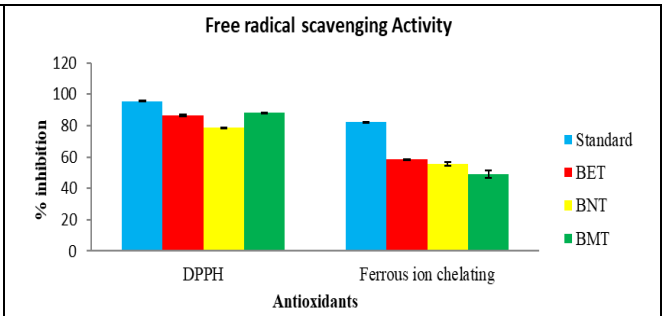


Figure 2: Free radical scavenging Activity

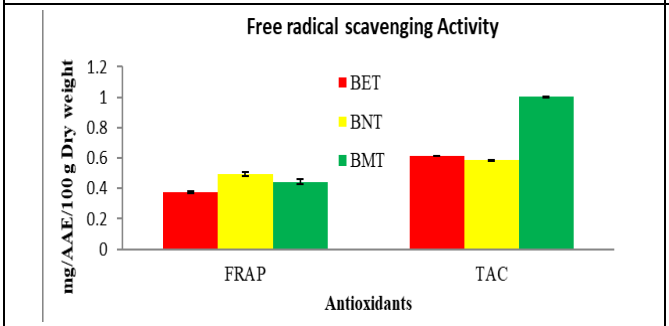


Figure 3: Free radical scavenging Activity

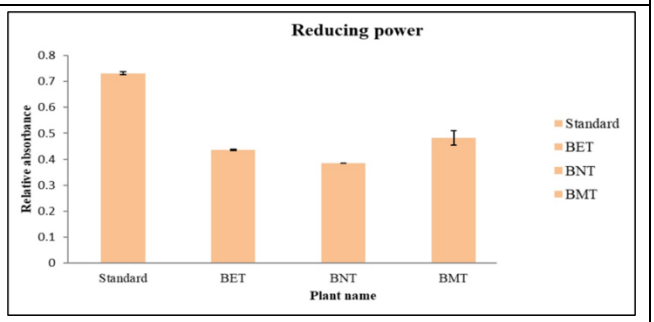


Figure 4: Reducing power

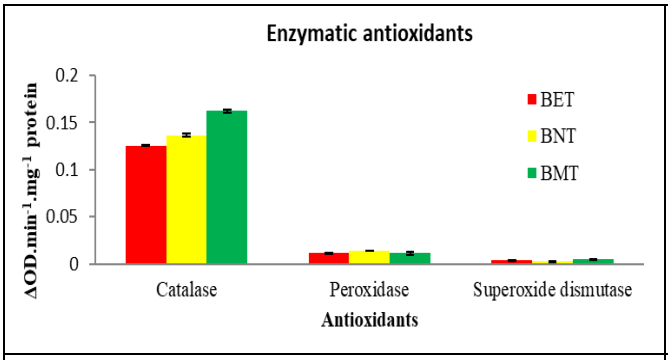


Figure 5: Enzymatic antioxidants

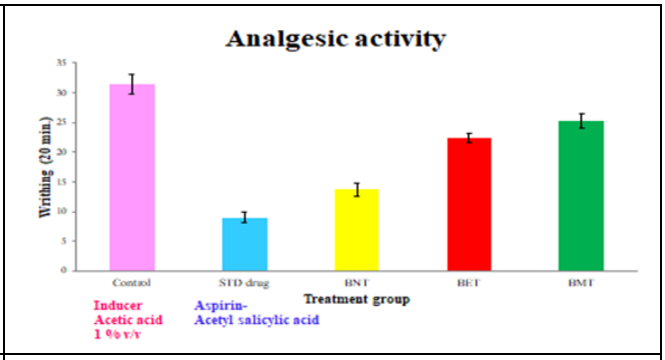


Figure 6: Analgesic activity

The findings of this investigation support the existence of common phytochemicals with analgesic and antioxidant properties, including flavonoids. Using the acetic acid-induced writhing response model, scientists have investigated the pain-relieving qualities of ethanol extracts derived from *Solena amplexicaulis* roots. The study's findings suggest that the *S. amplexicaulis* extract possesses promising analgesic and antioxidant properties[58]. The anti-inflammatory, analgesic, and antioxidant properties of polyphenols isolated from the ethanolic extract of *Brassica oleracea* var. capitata (cabbage)[59]. This research aims to provide scientific validation for the traditional medicinal applications of cabbage in treating various ailments. The researchers used mouse models demonstrating acute and subacute inflammatory conditions to assess the pain-relieving and anti-inflammatory properties of BOE when consumed orally or externally, and the antioxidant potential of orally administered BOE was assessed. These findings demonstrated that BOE contains high concentrations of phenolic compounds with strong antioxidant properties. These results support BOE's traditional application of BOE and highlight its potential for additional pharmacological research, suggesting that it may be a useful natural treatment for illnesses associated with inflammation. An uncommon plant on the Indian subcontinent is *Artabotrys hexapetalus* [60]. Numerous bioactive chemicals have been found in this plant and are used in folk medicine for a variety of therapeutic applications. It was discovered that the *A. hexapetalus* extract had a respectable level of tannins, flavonoids, and phenolic. In addition to its ability to reduce ferric chloride, this extract demonstrated good radical-scavenging properties. In analgesic tests, the extract showed excellent responses in writhing inhibition and elongation in tail withdrawal time. At both doses, the extract gradually reduced paw edema. There was also a noticeable decrease in the body temperature. Nice pharmaceutical potential was shown by the responses. Antioxidants are abundant in *A. hexapetalus* leaves, according to analysis, and the extract exhibited good pharmacological properties. This study evaluated the anti-inflammatory and pain-relieving effects of the Tibetan herb *Pterocephalus hookeri* (CB). Clarke) Höeck, using both ethanol and water based extracts. This study aimed to provide scientific evidence supporting the plant's traditional use in treating ailments such as rheumatoid arthritis, influenza, and common colds. The results indicate that these extracts demonstrate both systemic and localized analgesic properties as well as anti-inflammatory capabilities. These findings substantiate the historical application of this herb in the management of various pain and inflammation related conditions [61].

CONCLUSIONS

Brachystelma tubers are not only edible, but also rich in nutrients. The significant antioxidant capabilities of these tubers can be attributed to their polyphenol and other phytochemical element concentrations. The findings of the present study support the traditional use of the plant for alleviating pain, demonstrating that the tuber extract possesses strong analgesic and antioxidant properties. These effects may be linked to the presence of polyphenols as well as the concentrations of FRAP, DPPH, Ascorbic acid, carotenoids, peroxidase, and total antioxidants. The possible analgesic and antioxidant properties of the extract lend credence to this conventional assertion.

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The authors contributed as follows: S. R. More data collection and analysis: V. D. Jadhav performed review, supervision, literature search, initial draft writing, and subsequent revision and editing; A. B. Patil was responsible for conceptualization, visualization, and animal experimentation. All the authors have read and approved the final version of the manuscript.

Ethical approval:

This study was conducted in accordance with scientific ethical standards and practices, as affirmed by the researchers. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Tatya Saheb Kore College of Pharmacy, Warananagar, Kolhapur (M.S., 416113), India, with the reference number IAEC/TKCP.

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Dr. Varsha Dilip Jadhav, Professor in Botany is engaged in ethnobotanical studies and Nutraceutical analysis of wild plants of Maharashtra with special emphasis on Western Ghats. She has guided 14 students for Ph. D. and one student of M.Phil. Presently 4 students are working for their Ph.D. under her guidance. She has 95 research papers to her credit. She has made ethnobotanical survey of Kolhapur district and Forest Flora of Badami hills. Two new species of Portulaca (Portulacaceae) are to her credit. She is lifetime fellow and member of Indian Association of Angiosperm Taxonomy, Women Science Association of India, Life Member of mangrove society, Life member of Nutrition society, Member of Society of Ethnobotanist from Lucknow, and Member of International Botanical Congress. She has successfully completed 6 research projects funded by various governmental and Non-governmental agencies. Along with her students she has screened more than 100 wild edible plants for their nutraceutical values and 250 Ethno botanically important plants. Presently she is engaged in plant exploration, conservation and Bioprospecting of wild plants of Western Ghats. Recently she has presented paper in IBC at Madrid in July 2024.