

Phytochemical analysis, anti-oxidant and anti-inflammatory of *Belosynapsis vivipara* leaves and roots

Nitin A Sasane¹, Paresh S Gaikar², Sanjay L Gaikwad¹, Arun K Valvi², Gurumeet C Wadhawa^{2*}

¹ Mahatma Phule Arts, Science and Commerce College, Panvel, Maharashtra, India

² Rayat Shikshan Sanstha's, Karmaveer Bhaurao Patil College, Vashi, Navi Mumbai, Maharashtra, India

Abstract

Belosynapsis vivipara from the family Commelinaceae is a very rare plant. It is very good curative and work against various illness and infections. The plant part has large importance in Ayurveda. In this present study, roots and leaves were used for phytochemical analysis and their biological properties such as antioxidant and anti-inflammatory activity determined using a known protocol. Both the leaves and roots extract have shown the presence of various important active constituents as well as demonstrated better activity against the free radicals or antioxidant activity. Moreover, they have shown excellent anti-inflammatory activity.

Keywords: leaves, roots, *Belosynapsis vivipara*, DPPH and anti-inflammatory, radicals, scavenging

Introduction

Now a day's pollution is increased very rapidly. Due to pollution large number of free radicals are generated. Animals are exposed to these free radicals or as oxidizing agents and affect their metabolism very highly lifestyle and the new lifestyle and modern food habit lead to the generation of the free radicals. Most of the chemical species which contain reactive namely superoxide anion (O_2^-), nitric oxide (NO^*), oxygen species (ROS), the hydroxy group or peroxide radicals, or nitric oxide (NO) different nitrogenous species (RNS), peroxy nitrite anion ($ONOO^-$), agents like hypochlorous acid (HClO) and hydrogen peroxide ($HOOH$) [1]. Most of these free radicals like ROS and RNS are responsible for the lipid per oxidation, they also cause the protein cross-linking or attack the DNA, it results in various cataracts, atherosclerosis, chronic inflammation; other diseases like different diabetes, affect the cell damage causing cancer along with the various cardiovascular disorders liver and also affect the nerve [2]. Antioxidants used food in the processing industry to increase the life of the food, especially in lipid peroxidation generated by the free radicals as a consequence, increases food deterioration, discoloration, and nutritional losses, among others. Different antioxidants are prepared such as BHA, tertiary butyl hydroquinone, butylated hydroxytoluene (BHT), propyl-gallate are available [3]. These are some examples of plants that have been thoroughly considered in the few last years for their antioxidants. Anti-Inflammatory activity tissue to injury leads to inflammation. This is an example of a complex process. This inflammation is related to often related to cell damage and some pain in the cell includes occurrences such as lead to the swelling in the cells. Mostly the protein denaturation takes place, it basically involves the cascade of fluidic and cellular changes [4]. It leads to inflammation in the cell is due to the production of various radicals such as O_2 , OH and non-free radical species (H_2O_2) and this also leads to the extreme activation of phagocytes. They have a very powerful oxidizing action. Hence, the different agents present in the plant material increase the

radicals scavenging activity and the inflammatory activity [5].

Belosynapsis vivipara is a genus of mainly perennial plants in the family Commelinaceae, first described in 1871. It is native to Southeast Asia, the Indian Subcontinent and southern China. It is an epiphytic herb with creeping branches, which is 5-15 cm high covered with scattered rufous spreading hairs or glabrescent in the tender plants; rootstock small. Leaves are radical and cauline; radical leaves are 3-8 x 1-2 cm, sessile, linear or linear-lanceolate, base narrowed, apex acute or acuminate, covered with pilose hairs; cauline leaves 1-2 x 0.2-0.5 cm, sessile, ovate, or elliptic, apex acute, pilose. Peduncle with 2-4 flowers in an umbel, arising from the leaf axils, pilose, 2-bracteate. Sepals 3, 2-3 mm long, oblong, villous. Petals 3, white, connate to the middle [6].



Fig 1: *Belosynapsis vivipara* Plant

Most medicines are derived from the traditional way from time immemorial throughout the world and continue to provide new targets for remedies for many afflictions of mankind. The past couple of decades have seen a significant change in view regarding ethnopharmacological therapeutic claims of phytochemical. A great deal of effort therefore still focuses on identifying and using these phytochemicals, as a source of novel therapeutic molecules.

Materials and Methods

Collections of plant materials

Roots and leaves in large amounts *Belosynapsis vivipara* are collected in a nearby area of Lonavala, Maharashtra and identified by Dr. Arun Chandore Rayat Shikshan Santha's, Abasaheb Marathe Arts and Commerce, Science Rajapur Dist. Ratnagiri, Maharashtra, India. These samples are collected and washed with distilled water then these samples are dried. After complete drying, samples were pulverized and powered was obtained.

Phytochemical Analysis

Phytochemical testing carried given protocol and then other biological activity carried using the correct protocol. Phytochemistry of leaves Powder and root powder was carried out by using the following standard protocol. The leaves and roots extract of the *Belosynapsis vivipara* plant were named BV-L and BC-R respectively

Proteins

In the test for primary metabolites i.e. protein as per given protocol in the literature [7], the development of purple colour indicated the presence of protein. This test was positive for both BV-L and BV-R.

Polyterpenes and Sterols

Lieberman-Burchard's Reaction was used for the detection of polyterpenes and sterols with the standard protocol [89]. The brown ring formed at the junction of two liquid and greenish colour formation indicates sterols. The test was negative for both BV-L and BV-R.

Steroids

Salwoski's test was carried as per standard protocol for the detection of steroids [10], the formation of the reddish colour indicates the presence of the steroids. Salwoski's test for steroids was positive for BV-R and negative for BV-L.

Tannins

A Ferric chloride test was used for the identification of the tannins [10]. The test carried using the standard protocol, changing green colour to black indicates the existence of tannins. Ferric chloride test for tannins is positive for BV-R and negative for BV-L.

Anthraquinones

Borntrager's test was used for the identification of the anthraquinones derivatives. This test was carried using the standard protocol [11]. A pink, red or violet colour in the aqueous layer after shaken confirmations the occurrence of free anthraquinone. Borntrager's test was positive for both BV-L and BV-R.

Saponins

Frothing test is a particular test used for the identification of the saponins carried using the typical protocol [12]. In this test, the formation of honeycomb froth shows the existence of saponins. The frothing test was positive for BV-R and negative BV-L saponins detection.

Flavonoids

Flavonoids are important plant constituents test performed using the fixed protocol [13]. Pink or red colour shows the

existence of flavonoids. This test was positive for BV-R and negative for BV-L.

Phenolic nucleus

The sodium hydroxide test is particularly used for the identification of the phenolic nucleus. This test was carried using the fixed protocol [14]. In this test, the production of greenish-black colour indicates the presence of a phenolic nucleus. Sodium hydroxide test for detection of a phenolic nucleus was positive for both BV-L and BV-R.

Detection of Reducing Sugars

Reducing sugars were identified in given by the Fehling reagent, and confirmed by Tollens reagent test. To carry Fehling tests, 5-6 mL of the extract are added to 6 ml of Fehling's solution it forms red brick after min of a heating bath at 70 °C indicates a positive reaction. Tollens test consisted of adding around 4-5 mL of extract to 6 mL of the Tollens reagent result in the formation of a silver mirror [13]. This test for the plant extract was positive indicate the presence of a reducing agent. This test was positive for both BV-L and BV-R.

Alkaloids

Alkaloids are detected using the proper protocol [15, 16]. We tested the plant extract formation of Orange colour indicates the presence of alkaloid. This test was positive for BV-R and negative for BV-L.

Coumarins

In two test tubes, take 2 ml ethanolic solution obtained from each residue during extraction. In one of the test tubes, add 0.5 mL of 10% NaOH, heat both test tubes heated in a water bath until boiling. Cool both the test-tube test tube 4 ml of distilled water. If the liquid from the test tube in which was added the alkaline solution is transparent or more transparent compared to the control test tube liquid (without alkaline solution), it forms a faint yellow solution indicate the presence of Coumarins [7]. This test was positive for BV-R and negative for BV-L.

Detection of Tannins

Tannins was detected using the estimated protocol [17]. The formation of green colour shows the presence of tannins. This test was positive for BV-R and negative for BV-L.

Detection of Saponosides

This test was performed as per the reference this test required 6-7 ml of the sample formation of the froth indicate that saponosides present in the given sample [14]. This test is positive for BV-R and negative for BV-L.

Antioxidant Activity Determination

DPPH Scavenging Test: The percentage of the antioxidant present in the sample was determined using the typical protocol of the DPPH scavenging test. This test was carried out using the specified protocol [18, 19]. This test was carried out by preparing the various extracts of the plant material.

Study of anti-inflammatory activity (In-vitro models)

The anti-inflammatory activity of the different extracts was carried out using a slight modification of Mizushima and Kobayashi protocol with doses [20]. The albumin test method was used.

Results and Discussions

In the current study, the powder of *Belosynapsis vivipara* was exposed to preliminary phytochemical analysis. The extracts of plant samples shown the existence of different phytochemicals based on their polarity, extracting those plant metabolites of hydrophilic and hydrophobic nature. In this study, the *Belosynapsis vivipara* plant was screened for phytochemistry antioxidant as well as anti-inflammatory activity. The roots and leaves were subjected to the various phytochemical tests, the tests were positive for the anthraquinones derivatives, phenolic nucleus, proteins, saponosides and flavonoids, saponin, alkaloids, steroids, tannins, proteins, coumarins for BV-L and BV- respectively.

Antioxidant activity

The antioxidant and anti-inflammatory activities of the organic solvents of leaves and roots extract of *Belosynapsis vivipara* showed significant variations as shown in Table: 1 to Table: 4. Fig. 2. displayed the variation of antioxidant activity of BV-L and BV-R in BHT, ethanol extract, chloroform extract and aqueous pure plant extract.

Table 1: Antioxidant activity of BV-L

Extract Conc. mg/ml	BHT	Ethanol extract	Chloroform extract	Aqueous Pure plant extract
0.05	42.30	16.45	30.49	40.37
0.1	48.60	32.55	40.87	44.60
0.2	54.24	43.53	43.80	52.30
0.3	60.14	48.00	46.60	56.50

Table: 1 revealed that the ethanol fraction of the plant material having good antioxidant activity, while chloroform and pure plant extract exhibits less activity.

Table 2: Antioxidant activity of BV-R

Extract Conc. mg/ml	BHT	Ethanol extract	Chloroform extract	Aqueous Pure plant extract
0.05	42.30	18.42	25.40	38.40
0.1	48.60	32.30	39.60	47.80
0.2	54.24	46.50	32.35	52.35
0.3	60.57	53.60	48.40	58.60

Table. 2 displayed that the ethanol extract of the roots shows excellent activity compared with the standard antioxidant BHT, ethanol, pure plant extract and chloroform extract. After comparison of ethanol, pure plant and chloroform extract antioxidant properties of BV-R and BV-L. BV-R has better antioxidant properties than BV-L. The ethanol extract of the roots and leaves shows excellent activity against the standard antioxidant agents. This antioxidant activity, we can obtain very effectively from the roots and leaves of the plant. This can help us in the curing of various diseases like cardiovascular disorders, cancer, aging, rheumatoid arthritis and diabetes. This antioxidant activity was due to the phenolic components in the roots and leaves of *Belosynapsis vivipara* and the presence of various phytochemicals in the plant extract.

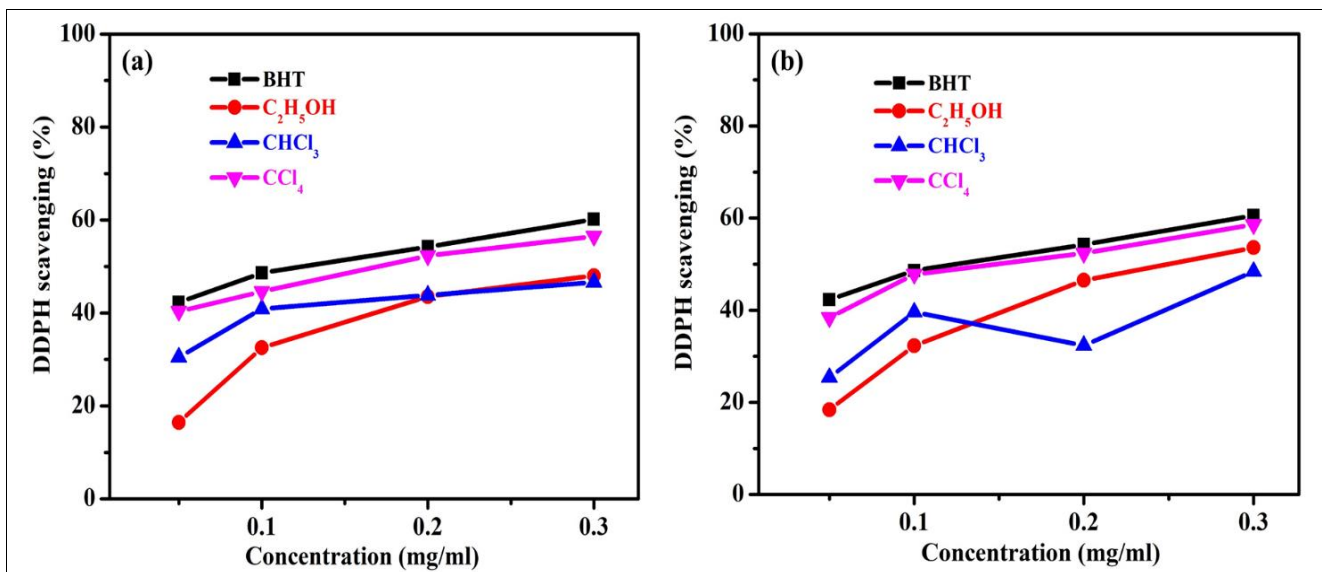


Fig 2: Antioxidant activity of BV-L and BV-R a) DPPH radical activity of BV-L b) DPPH radical activity of BV-R

Anti-inflammatory activity

Fig. 3 shows the comparative study of anti-inflammatory activity

of BV-L and BV-R with Ibuprofen, ethanol, chloroform and pure plant extract

Table 3: Anti-inflammatory activity of BV-L

Anti-inflammatory activity	Dose (mg / kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	100mg/ kg	0.098	Nil
Standard (Ibuprofen)	200mg/kg	0.20	98
Ethanol extract	200mg/kg	0.18	78
Chloroform extract	200mg/kg	0.12	66
Pure Plant	200mg/kg	0.14	88

The anti-inflammatory activity of BV-L was studied with Ibuprofen, ethanol, chloroform and pure plant extract. Table

3. Displayed the anti-inflammatory activity of BV-L. The activity is compared with the Ibuprofen as standard. This

extract shows good results at the same time ethanol extract and pure plant extract showed good activity while the chloroform shown pure activity.

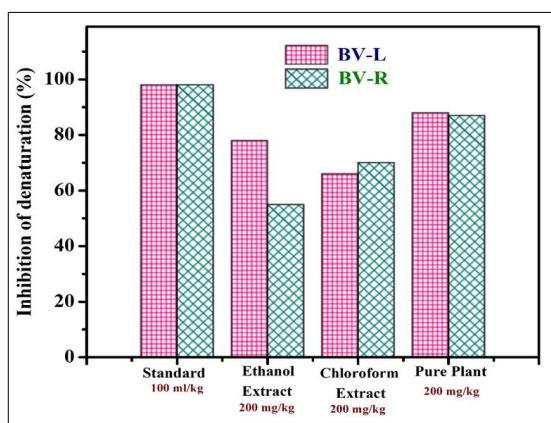


Fig 3: % inhibition of BV-L and BV-R for standard, Ethanol, Chloroform, and Pure plant extracts

Table 4. Displayed the anti-inflammatory activity of BV-R. Pure plant extract shown better anti-inflammatory activity than ethanol and chloroform.

Table 4: Anti-inflammatory activity of BV-R

Anti-inflammatory activity	Dose (mg/kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	100mg/kg	0.098	Nil
Standard (Ibuprofen)	200mg/kg	0.20	98
Ethanol extract	200mg/kg	0.16	55
Chloroform extract	200mg/kg	0.11	70
Pure Plant	200mg/kg	0.13	87

The result of anti-inflammatory shows good albumin denaturation and membrane stabilization. The ethanol extract shows a very good anti-inflammatory activity, so we can use this plant as a good anti-inflammatory agent. This indicates this plant has traditional use and good medicinal activity. The roots and leaves of *Belosynapsis vivipara* exhibited good antioxidant and anti-inflammatory activity.

Conclusion

The roots and leaves extract of the *Belosynapsis vivipara* plant shown very good antioxidant and anti-inflammatory activity due to the presence of the various phytochemicals in the plant extract. *Belosynapsis vivipara* roots and leaves extract can be used as a good antioxidant as well as an anti-inflammatory agent. We can increase our strength of the Ayurveda by use of various plant cultivation in nature.

Conflict of Interest

The authors declare no conflict of interest.

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