



Research article

Cytomorphological, Palynological and Phytochemical study of two species of Genus *Bacopa* (Scrophulariaceae) from the state of Punjab, India

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Abstract: Two species of the genus *Bacopa* (*B. monnieri* and *B. procumbens*) were studied for examination of cytomorphological and palynological characteristics as well as the screening of phytochemical from the Malwa region of Punjab during the years 2019, 2020 and 2021. Primarily both species are differentiated based on morphological features then identity was authenticated by examination of chromosome number. There is a great difference in chromosome number between the two species (*B. monnieri*, $2n=64$; *B. procumbens*, $2n=22$). Pollen size of *B. monnieri* is larger ($22.5 \times 21.25 \mu\text{m}$ to $27.5 \times 25 \mu\text{m}$) with less viability (77.87%) whereas in case of *B. procumbens* pollen size is small ($15 \times 13.75 \mu\text{m}$ to $17.5 \times 16.25 \mu\text{m}$) with high viability (88.75%). The phytochemicals of both species are almost similar with minor variations. This study will be helpful for scientists in the identification of these important medicinal plant species. The phytochemical profile throws light on medicinally important compounds which will be utilized by pharmaceutical departments for the development of appropriate drugs.

Keywords: Chromosome - *Bacopa* - Pollens - Phytochemical - Weed.

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INTRODUCTION

Bacopa Aubl. is the largest genus of the tribe Gratiioleae with 60 species (Fischer 2004). Plants of this genus annual to perennial; prostrate, erect; succulent leaves; aquatic or submerged; herbs (Sosa *et al.* 2018). Morphological features (habit, stem, leaf, flower, fruit and seeds) of plant species are usually important in identification of species. It is a classical tool used by scientists for the identification of organisms.

After the morphological study, chromosome number is also used for the identification of species. According to Sidhu & Singh (2021a) every species have its specific chromosome number. Therefore the chromosomal study is useful for the identification of species. Similarly, pollen size throws light on the difference between closely related species because it varies from species to species. Pollen viability is directly related with chances of pollination and fertilization. Therefore, it is responsible for final seed setting of the plant species.

Phytochemicals or biochemical are the chemical compounds which are synthesized by the plants as primary or secondary metabolites. They consist of carbohydrates, proteins, glucose, alkaloids, flavonoids, tannins, terpenoids etc. These phytoconstituents are responsible for medicinal properties of the respective plant species (Kabila *et al.* 2020).

Punjab state is situated in the Northern part of India and it has three major regions *viz.* Majha, Malwa and Doaba. Few reports about the floristics are available from the state of Punjab (Sharma & Bir 1978, Meenakshi & Sharma 1985, Sharma *et al.* 1987, Sharma 1990, Sidhu 1991, Singh & Singh 2019, Singh & Singh 2020, Sidhu & Singh 2020, Sidhu & Singh 2021a, Sidhu & Singh 2021b). But information about cytomorphological, palynological and phytochemicals species of the genus *Bacopa* is incomplete. Keeping this in view present study has been undertaken.

Present investigation has been undertaken during the years 2019, 2020 and 2021 in Malwa region of Punjab, India. Two species of *Bacopa* (*B. monnieri* and *B. procumbens*) were collected from *kharif* crops as a weed.

Morphological features, chromosome number and pollen size were analyzed for proper identification of *Bacopa* species. Pollen viability was examined for estimation of capacity of final seed setting of species. Phytochemicals of plant species are responsible for development of valuable drugs. Therefore phytochemicals of leaves of *Bacopa monnieri* and *Bacopa procumbens* are also screened.

MATERIALS AND METHODS

Study area

Punjab is an agricultural state of the India present in the Northern part of the Country. Agriculture is the economic backbone of the state. The present study has been carried out in different *Kharif* crops of the Malwa region during the years 2019, 2020 and 2021. The temperature of the study area varies with seasons (3°C to 47°C). Soil is sandy to sandy loam.

Morphological, chromosomal and pollen Study

Morphological features such as leaf, stem, flower, stamens, stigma, fruit, seeds *etc.* were examined for preliminary identification. For meiotic study, young flower buds were collected and fixed in the fixative (ethanol 3: glacial acetic acid 1) for 24 hours then shifted to 70% ethanol. For the preparation slide, anthers were taken from bud, added 1-2 drops of acetocarmine and crushed. Debris were removed by using a needle then the material was covered with a cover slip and warmed. After that, the slide was pressed into two folds of filter paper. Then observed under the Digital microscope. Pollen viability was carried out through stainability tests. For this process mature anthers were taken on the slide and squashed in glycerol acetocarmine (1:1) mixture and stood for 1 day. After 48 hours some pollens were stained and some were unstained. Stained Pollen were fertile and poorly unstained pollens were sterile. The percentage of viable pollens was calculated (Stained Pollens / Total Pollens x 100).

Phytochemical analysis

Plant material: Plant material (leaves) was collected during *Kharif* season from different sites of the study area.

Plant material was washed and dries at room temperature. After proper drying plant material was grinded using an electric grinder.

Preparation of extracts: Plant extract were prepared in three solvent (water, ethanol and hexane) (Table.1).

Table 1. Methods of preparation of extracts.

S.N.	Extract	Method
1	Aqueous	20 gm powder (plant sample) + 100 ml distilled water + orbital shaker (24 hours) + filter (muslin cloth) + filter (Whatman No.1 filter paper)
2	Ethanol	10 gm powder + 130 ml (ethanol) + Soxhlet apparatus (temperature 60°C)
3	Hexane	10 gm powder + 130 ml (hexane) + Soxhlet apparatus (temperature 35°C)

Analysis of Phytochemicals

Phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, reducing sugar, saponins, steriods, terpenoids, tannins *etc.* were screened using standard procedures (Harborne 1973, Trease & Evans 1989, Sofowora 1993, Kokate 1994, Harborne 1998, Evans 2002, Kulkarni 2008, Devmurari 2010, Idu & Igeleke 2012, Ugochukwu *et al.* 2013, Sidhu & Sharma 2016, Oncho *et al.* 2021).

RESULTS AND DISCUSSION

Bacopa monnieri (L.) Wetts. is annual prostrate herb. The stem is branched, herbaceous, fleshy, cylinder and green. Leaves are opposite, linear, fleshy and rounded at the end. Flowers bisexual, complete; corolla white to bluish-white; stamens-4, anthers black with long filament. Fruits linear capsule with two bracts. Seeds are numerous, small, shiny and black in color (Fig. 1A). This species possess $2n=64$ chromosome number. Thirty-two bivalents were observed at diakinesis (Fig. 2A). Present results of chromosome number is the agreement with Raghavan (1959), Lewis *et al.* (1962), Samadder *et al.* (2012), Christopher (2016) and Samanta *et al.* (2019). Only Carr (1978) reported $n=34$ chromosomes in this species in his study from Hawaiian. This may be due to geographical restrictions. The Pollen size of this species is $22.5 \times 21.25 \mu\text{m}$ to $27.5 \times 25 \mu\text{m}$. The pollen viability of this species is 77.87% (Fig. 3A,B).

Bacopa procumbens (Mill) Greenm. (Syn. *Mecardonia procumbens* (Mill.) Small.) is an annual, prostrate and branched herb. The stem is 4-angled, simple or branching from the base, decumbent to ascending to prostrate and creeping, glabrous to hairy. Leaves sessile to somewhat amplexicaul, ovate, obtuse to round at the apex, margins crenate, glabrous. Flowers yellow. Capsule ovoid to long, brown, glabrous, included in the calyx.

Seeds are small, rounded, shiny and black in color (Fig. 1B). *Bacopa procumbens* is diploid in nature and has shown eleven bivalents at metaphase-I (Fig. 2B). The present findings of chromosome is consonance with Sinha (1987). Sosa *et al.* (2016) also reported the same number of chromosomes ($n=11$) from Argentina. This study has shown this species is genetically stable throughout the world. The size of pollens are ranges between $15 \times 13.75 \mu\text{m}$ to $17.5 \times 16.25 \mu\text{m}$ and viability of pollen grains is 88.75% (Fig. 3C,D). Both *Bacopa monnieri* and *B. procumbens* are member of the same family as well as same the genus but has shown a difference in pollen size. This dissimilarity between the two species is may be due to differences in chromosome number.



Figure 1. Habit of *Bacopa* species: **A**, *Bacopa monnieri* (L.) Wetts.; **B**, *Bacopa procumbens* (Mill) Greenm.

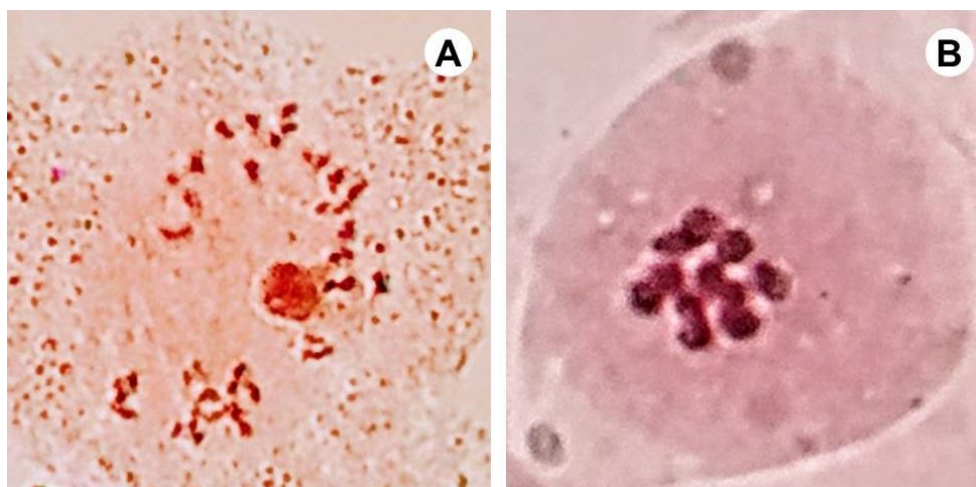


Figure 2. Chromosome number of *Bacopa* species: **A**, *Bacopa monnieri* (L.) Wetts. ($n=32$); **B**, *Bacopa procumbens* (Mill) Greenm. ($n=11$).

Leaves of both species of the genus *Bacopa* shown positive results for twenty-one phytochemicals (Alkaloids, Amino acids, Anthocyanin, Betaxanthin, Carbohydrates, Coumarins, Flavonoids, Glycosides, Gums and mucilage, Oxalate, Phenols, Phlobatannins, Proteins, Quinones, Reducing sugars, Resin, Saponins, Starch, Steroids, Tannins and Terpenoids) in variable concentrations *i. e.* presence (+) or traces (\pm) (Table 2.). Alkaloids were reported in ethanol extract of both species and only aqueous extract of *B. monnieri*. Alkaloids have antibacterial, anticancerous, anti-inflammatory, antidiabetic and insecticidal properties (Qiu *et al.* 2014). Amino acids and proteins were recorded in aqueous extract of both species but proteins were only present in the ethanol extract of both species. Amino acids usually regulates blood pressure level (Rose 2019). Anthocyanins were also observed only in the aqueous and ethanol extract of both species. Anthocyanin protects important biomolecules (DNA, proteins and lipids) from oxidative damage (Martin *et al.* 2017). It is also useful in the treatment of lymphoma, pulmonary carcinomas, gastric adenocarcinoma and ovarian carcinoma (Bagchi *et al.* 2002). Betaxanthins was present in ethanol and hexane extracts of both species. It has antioxidant and antitumor properties (Lechner & Stoner 2019, Vijaya & Thangaraj 2020). Carbohydrates, glycosides and starch were

present in all the extracts of both species whereas reducing sugars were not seen in aqueous and ethanol extract of both plants. Carbohydrates and glycosides have antibacterial and anticancerous properties and also suitable for the treatment of heart diseases (Anandhi *et al.* 2014, Kokkinidou *et al.* 2018). Coumarins and flavonoids were recorded in all extracts of both species in variable concentrations. Sodium hydroxide (NaOH) test shows the best results for flavonoids. According to Kapoor (2013), coumarins have anti-coagulant, antioxidant, analgesic, anti-neurodegenerative and anti-inflammatory properties. Flavonoids act as antitumor and antiviral agents (Kumar & Pandey 2013). Gums and mucilages are water-soluble polysaccharides and useful as anti-inflammatory, dental adhesives, laxative and emulsifying agent (Upadhyay 2017). They show positive results in the aqueous and ethanol extracts of both species. Resins were present in the ethanol extract of both species and the aqueous extract of *Bacopa monneiri*. Oxalates were observed in ethanol extract of both species, aqueous extract of *Bacopa procumbens* and hexane extract of *B. monneiri*. According to Huang *et al.* (2015) leaves of plants possess maximum concentration of oxalates than the root and stem.

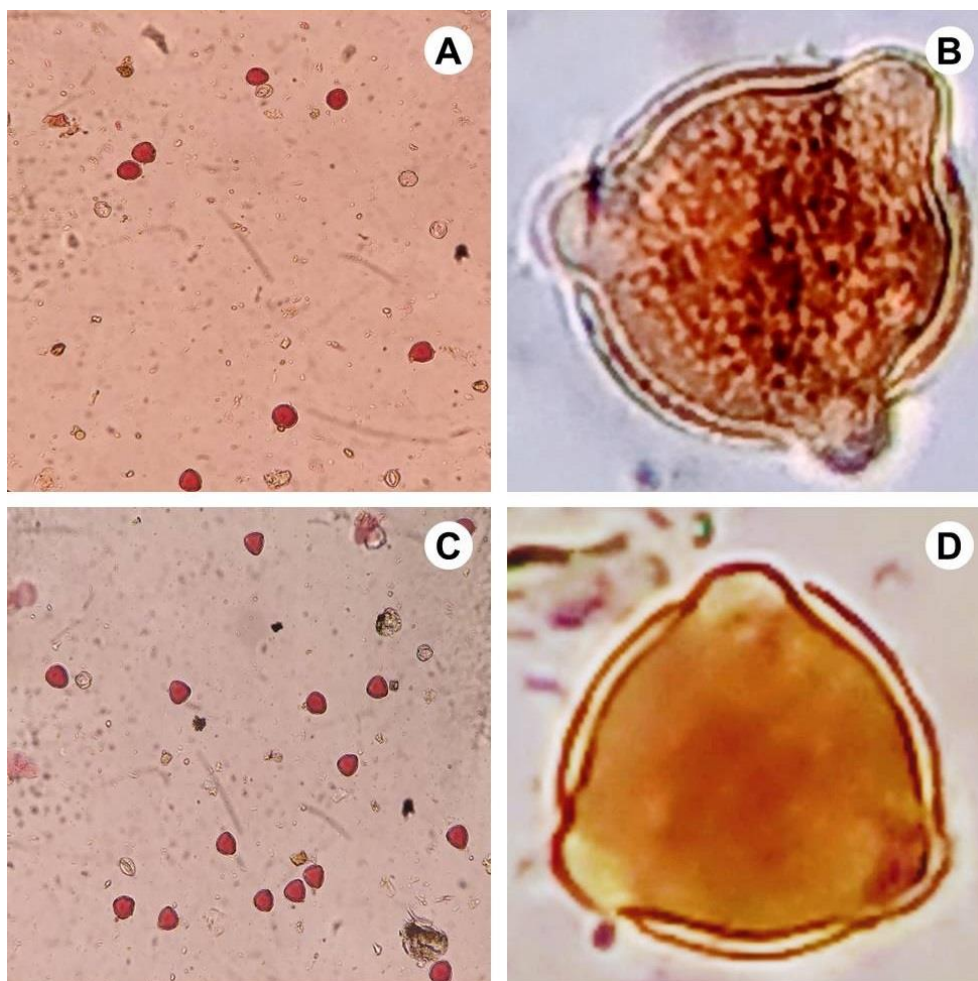


Figure 3. Pollen viability and pollen grain: **A-B**, *Bacopa monneiri* (L.) Wetts.; **C-D**, *Bacopa procumbens* (Mill) Greenm.

Phenols are a protective agent for many diseases such as osteoporosis, pancreatitis, gastrointestinal problems, lung damage and neurodegenerative diseases (Fujiki *et al.* 2015, Xiao & Hogger 2015). It is present in aqueous and ethanol extract of both species and hexane extract of *Bacopa procumbens*. Phlobatannins were only recorded in an ethanol extract of both species. Only the hexane extract of *Bacopa procumbens* showed positive results for quinones. Quinones have antioxidant properties and also protects against cardiovascular diseases (Pepe *et al.* 2007, Nageswara Rao *et al.* 2008). The steroid is a cholesterol-reducing phytochemical found in many plants (Shah *et al.* 2009). It is only observed in the hexane extract of *Bacopa monneiri*. Saponins were only observed in the aqueous extract of both species. They have anti-inflammatory, antiviral, antifungal, insecticidal, molluscicidal, piscidal and anti-bacterial properties (Mbaveng *et al.* 2018). Tannins and terpenoids were reported in all the extracts of both species. Tannins have important physiological activities such as free radical scavenging and inhibition of pro-oxidative enzymes and lipid peroxidation (Koleckar *et al.* 2008). Terpenoids reduces diastolic blood pressure and sugar level in the blood (Grace *et al.* 2014). All phytochemicals have medicinal properties and are useful against various kinds of diseases.

Table 2. List of phytochemicals in *Bacopa* species.

S.N.	Test	<i>B. monnieri</i>			<i>B. procumbens</i>		
		Aq	Et	He	Aq	Et	He
1	Alkaloids	±	+	-	-	±	-
2	Amino Acids	±	-	-	±	-	-
3	Anthocyanin	+	+	-	+	+	-
4	Betaxanthin	-	+	+	-	+	+
5	Carbohydrates	+	+	+	+	+	±
6	Coumarins	±	+	±	+	+	+
7	Flavonoids (NaOH) (H ₂ SO ₄)	±	+	±	±	+	+
		-	-	-	-	-	-
8	Glycosides	+	+	±	+	+	±
9	Gums and mucilage	+	±	-	+	±	-
10	Oxalate	-	+	±	±	+	-
11	Phenols	+	+	-	+	+	±
12	Phlobatannins	-	+	-	-	±	-
13	Proteins	±	+	-	±	+	-
14	Quinones	-	-	-	-	-	±
15	Reducing sugars	±	+	-	+	+	-
16	Resin	±	±	-	-	±	-
17	Saponins	+	-	-	+	-	-
18	Starch	+	+	+	+	+	+
19	Steroids	-	-	±	-	-	-
20	Tannins (FeCl ₃) (KOH)	+	+	-	+	+	±
		-	-	±	-	-	±
21	Terpenoids	±	+	±	±	+	±

Note: Aq, Aqueous; Et, Ethanol; He, Hexane; +, Present; -, Absent; ±, Trace.

CONCLUSION

Both species are medicinally important species therefore present study was planned for accurate identification. Morphologically, *Bacopa monnieri* is easily differentiated from *B. procumbens* on the basis of colour of flower and nature of the stem. Cytologically, one species possess $2n=64$ (*Bacopa monnieri*) and another possess $2n=22$ (*B. procumbens*) chromosome number. The nature of phytochemicals of both the species are almost similar. Therefore, both species will be useful in development of drugs. This study will be useful for taxonomists, cytologists, palynologists etc. for identification of species and pharmacists for development of drugs.

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