Chapter-IV

DISCUSSION
Discussion

Punarnava is an important drug used in folk medicine, indigenous systems of medicine and even in modern medicine. The use of Punarnava is also mentioned in RigVeda, Matsya Purana, Agni Purana (Guha Bakshi et al., 1999). The authorities of Ayurveda viz. Vagbhatta, Charak, Sushruta, and Chakradatta have attributed different properties to this drug. Vagbhatta (Gadre, 1956) has attributed antiinflammatory property to Punarnava. The use of Punarnava is also mentioned in ‘Bhava Prakash’ and in different ‘nighantus’ viz. Brihan nighantu (Pade, 1914), Nighantu Kalpadrum (Trivedi, 1932), Dhanwantariya nighantu (Kamat and Mahajan, 1972), Rajnighantu (Bose, 1984) etc. In different nighantus Punarnava has been recommended for kapha disorders, swellings, eye diseases, cough, anemia, heart diseases, nervous system disorders, etc. In nighantu Kalpadrum three different varieties of Punarnava viz. Shwet, Rakta and Neel have been differentiated and to each variety specific properties are attributed. In Rajnighantu, Rakta Punarnava is specifically recommended for Kapha disorders while Shwet Punarnava is for swellings, eye diseases, cough and anemia.

There is controversy regarding the correct identification, use and potency of two varieties of Punarnava viz. Rakta Punarnava and Shwet Punarnava (Chopra et al., 1940; Chakravarty, 1942; Deshmukh et al., 1957; Saha and Krishnamurthy, 1962; Nair, 1967). Moreover, Singh and Udupa (1972 a) mentioned that Punarnava and Varshabhu are two different plants and Punarnava can be equated with B. diffusa L.
and the characteristics of ‘Varshabhu’ tally with \( T. \) portulacatum \( L. \).

The species \( B. \) diffusa \( L. \) is regarded as Rakta Punarnava, while, 
\( B. \) erecta \( L. \), \( B. \) punarnava Saha & Krish., \( B. \) verticillata Poir. and 
\( T. \) portulacastrum \( L. \) have been equated with Shwet Punarnava. However, to \( B. \) verticillata Poir., no important medicinal property is 
attributed (Saha and Krishamurthy, 1962) and \( B. \) punarnava 
Saha & Krish. has been proved to be \( B. \) erecta \( L. \) (Nair, 1967). In the 
present investigation three species of \( Boerhaavia \) viz. \( B. \) diffusa \( L. \), 
\( B. \) erecta \( L. \) and \( B. \) repanda Willd. and one species of \( Trianthema \), 
\( T. \) portulacastrum \( L. \) have been worked out due to their availability in 
the study area (Maharashtra state), their medicinal applications 
(\( B. \) diffusa \( L. \), \( B. \) repanda Willd. and \( T. \) portulacastrum \( L. \)) and use of 
some species (\( T. \) portulacastrum and \( B. \) erecta \( L. \)) as substitutes or 
adulterant in market samples of Punarnava. Review of literature revealed 
that the comparative account of all these four species has not been 
worked out. In view of these facts, in the present investigation four 
Punarnava species viz. \( B. \) diffusa \( L. \), \( B. \) erecta \( L. \), \( B. \) repanda Willd. and 
\( T. \) portulacastrum \( L. \) have been studied by pharmacognostical and 
pharmacological methods. In the present investigation an attempt has been 
made to develop comparative account of various Punarnava species to 
study Rakta Punarnava and Shwet Punarnava through the detailed 
morphological, microscopical, chemical and biological investigations.

The study of the distribution of a medicinal plant is an important 
aspect because it throws light on the availability and conditions required 
for the growth of a particular plant. The distribution of Punarnava species
viz. B. *diffusa* L., B. *erecta* L., B. *repanda* Willd. and *T. portulacastrum* L. is reported in different floras viz. Flora of the Presidency of Bombay (Cooke, 1902, 1967 Repr.), Flora of Purandhar (Santapau, 1957), Flora of Tamil Nadu Carnatic (Matthew, 1983), Flora of Himachal Pradesh (Chawdhari and Wadhawa, 1984), Flora of Saurashtra (Bole and Pathak, 1988), Flora of Akola District, Maharashtra (Kamble and Pradhan, 1988), Flora of Eastern Karnataka (Singh, 1988), Flora of Sawantwadi, Maharashtra (Almeida, 1990), Flora of Nasik District, Maharashtra State (Laxminarasimhan and Sharma, 1991), Flora of Rajasthan (Shetty and Singh, 1991), Flora of Mahabaleshwar and Adjoining Maharashtra (Deshpande *et al.*, 1993), Flora of Yawatmal District (Karthikeyan and Kumar, 1993), Flora of Maharashtra State (Singh *et al.*, 2001), etc. The records from these floras revealed that *B. diffusa* L. and *T. portulacastrum* L. are widely distributed in almost all the regions of India. However, in very few floristic accounts, the distribution of *B. erecta* L. and *B. repanda* Willd. is reported. In the present investigation, it has been observed that *B. diffusa* L., *B. erecta* L. and *T. portulacastrum* L. are widely distributed in Maharashtra state, however, *B. repanda* Willd. was found comparatively infrequent in different districts of Maharashtra viz. Dhule, Nasik, Jalgoan, Sindhudurg, Raigadth and Pune.

For procuring genuine material of medicinal plant and for the commercial exploitation there is a need for the standardization of the multiplication (propagation) techniques. The literature survey also revealed that almost no work has been done on this aspect.

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In view of this, in the present investigation, preliminary work on propagation has been carried out by employing stem cutting and seed methods. The Seed germination studies under *in vitro* and in field conditions showed 87.5%, 90.0% and 69.0%, 70.0% seed germination respectively for *B. erecta* L. and *B. repanda* Willd. The high percentage of seed germination in *B. erecta* L. indicates that the propagation by seeds will be more appropriate method for this plant. However, vegetative propagation by stem cutting method did not show encouraging results, since very low success rate, 10.0-19.5% is recorded in Punarnava species.

The literature survey showed that there is a lot of controversy about the taxonomical identity of Punarnava species. Hooker (1885) recorded six species of *Boerhaavia* viz. *B. repens* L., *B. crispa* Heyne, *B. repanda* Willd., *B. verticillata* Poir., *B. fruticosa* Dalz. and *B. elegans* Choisy. While Cooke (1902, 1967 Repr.) recorded five species of *Boerhaavia* viz. *B. diffusa* L., *B. repanda* Willd., *B. verticillata* Poir., *B. fruticosa* Dalz. and *B. elegans* Choisy. and five species of *Trianthema* viz. *T. monogyna* L., *T. decandra* L., *T. hydaspica* Edgew., *T. pentandra* (L.) Jeffrey. and *T. triquetra* Rott. & Willd. However, none of them reported *B. erecta* L. as this is introduced later from America as a weed. The literature survey also showed that there is difference of opinion among the taxonomists about the status of *B. procumbens* Banks ex. Roxb., *B. repens* L. and *B. diffusa* L. Singh (1988) treated these three species as con-specific due to high variability. However, Shetty and Singh (1991) considered these as
separate species. Matthew (1983) and Singh et al. (2001) differentiated two separate genera *Boerhaavia* and *Commicarpus* on the basis of fruit length and number of ribs present on it. They equated *B. repanda* Willd. with *Commicarpus chinensis* (L.) Hemeirl. Even *T. monogyna* L. has been equated with *T. portulacastrum* L.

It was realized that for the correct identification of Punarnava species there is a need for detailed taxonomical studies and morphological investigations have been carried out in the present investigation. The descriptions from the different plant floras showed that the classification of Punarnava species is mainly based on habit and fruit characteristics. In the present investigations morphological parameters such as habit; plant height (or spread); root type, root colour, root dimensions; stem characteristics; leaf type, thickness, shape, apex, margin, dimensions of large and small leaves; inflorescence type, panicle length; flower colour, perianth length, stamen number, characteristics of stamen; characteristics of style, carpel number, length, number of locules, type of placentation; type of fruits, fruit dimensions; number of seeds/g and dimensions of seeds have been recorded for differentiation of Punarnava species. It was observed that the root system, colour and size of *T. portulacastrum* L. root is quite distinct from other Punarnava species. The inflorescence of all the four species is quite characteristic. The length of perianth of *B. repanda* Willd. is large (0.78 cm) as compared with *B. erecta* L. (0.12 cm). In general, the morphological investigation revealed that all the four Punarnava species are quite distinct and can be differentiated and identified on the basis of
exomorphic features. In *T. portulacastrum* L. 10-20 stamens while in *Boerhaavia* species (*B. diffusa* L., *B. erecta* L. and *B. repanda* Willd.) 1-4 stamens are recorded. The fruit length is >0.5 cm and <0.2 cm respectively for *B. repanda* Willd. and *B. diffusa* L. In *B. diffusa* L. the prostrate habit while in *B. erecta* L. erect habit is observed. Based on morphological features Punarnava species are differentiated and identified. For easy and rapid identification of Punarnava species the identification key has been prepared in the present investigation.

The microscopical investigations are an integral part of the standardization of the drugs. Even in Pharmacopoeias, the microscopical standards of the drug are given. In view of this, detailed microscopical studies of pollens, stomata, trichomes, starch grains and crystals along with as well as detailed anatomical studies of root, stem and leaf of all four Punarnava species have been carried out. The average diameter of *B. diffusa* L. pollen is recorded higher (76.11 μ) as compared to the pollen diameter (66.7 μ) reported by Nayar (1990). However, Phatak (1994) recorded average pollen diameter 58.3 μ which is quite small as compared to the observed size in the present investigations. But the other characteristics such as shape, wall thickness and ornamentations are comparable. The pollen characteristics reported by Kannabiran (1973 a) for *B. punarnava* Saha and Krish. shows similarity with the pollens of *B. repanda* Willd. instead of *B. erecta* L. The range of pollen diameter he reported is 110.0-130.0 μ for *B. punarnava* Saha and Krish. while in the present investigation 52.0-90.0 μ diameter is found for *B. erecta* L. However, in *B. repanda* Willd.
it is 101.0-135.0 μ. This clearly shows that the size of pollens of
*B. punarnava* Saha and Krish. is comparable with the *B. repanda* L.
The pollens of *B. diffusa* L., *B. erecta* L. and *B. repanda* Willd. are
porate where as in *T. portulacastrum* L. the pollens are colpate with
comparatively smaller in diameter, 55.0µ (45.0-90.0 µ). The presence of
colpa in pollens is the distinct feature of *T. portulacastrum* L. Based on
the pollen characteristics identification key has been prepared.
Pollen analysis serves as a tool for the identification and differentiatation of
closely related species.

The peculiarities of trichomes of some Punarnava species are
reported by Metcalfe and Chalk (1950), Datta and Mukerji (1952),
epidermis of *B. erecta* L. showed 1-4 and *B. repanda* Willd. showed
1-6 stalk cells. While the dimensions recorded are 135.7 X 29.6 μ and
154.4 X 26.8 μ respectively for *B. erecta* L. and *B. repanda* Willd.
In *T. portulacastrum* L. non-glandular (eglandular) trichomes are seen on
lower epidermis, while, in *B. diffusa* L., *B. erecta* L. and *B. repanda*
Willd. on both the epidermal surfaces glandular trichomes are present.

The analysis of the stomatal and epidermal cells of leaf is used as
criterion for differentiation of the closely related species. Paliwal *et al.*
(1980) reported anomocytic type of stomata in *B. diffusa* L., while,
Anonymous (1989) recorded anomocytic and anisocytic type of stomata
in *B. diffusa* L. In the present investigation both the types of stomata,
anomocytic and anisocytic are recorded in *B. diffusa* L., however,
anisocytic stomata are quite infrequent. Datta and Mukerji (1952) also
reported anomocytic and anisocytic stomata in *T. portulacastrum* L. however, in the present investigation only anomocytic stomata are recorded on both the surfaces of this species. The size of stomata (upper epidermis) is reported 32.8 X 20.2 μ in *T. portulacastrum* L. (Datta and Mukerji, 1952) however, it is recorded high 41.2 X 25.1 μ in the present investigation. In general, it is observed that upper surface stomata are larger in size than the lower epidermis. The outline and dimensions of epidermal cells in surface view also serve as diagnostic feature for differentiation of closely related Punarnava species. Kannabiran (1973 a) reported straight walled epidermal cells on upper epidermis of *B. punarnava* Saha and Krish. The same type of epidermal cells were observed in *B. diffusa* L. and *B. erecta* L. While in *B. repanda* Willd. the characteristic wavy outlined epidermal cells are observed. The dimensions of epidermal cells (upper epidermis) are quite distinct in Punarnava species. For *B. repanda* Willd. the epidermal cell size is small 40.85 X 31.1 μ while for *B. diffusa* L. it is 74.7 X 56.7 μ. The trichomes, epidermal and stomatal characteristics serve as a diagnostic feature for Punarnava species. Thus identification key has been prepared based on the stomatal and epidermal features.

The detailed anatomical studies of root, stem and leaf are carried out as these are essential in the standardization of the drugs. Even in Pharmacopoeias descriptions of the microscopic characteristics of the drugs are given. Maheshwari (1930), Datta and Mukerji (1952), Deshmukh *et al.* (1957), Singh and Udupa (1972 a), Kannabiran (1973 a) and Surange *et al.* (1977) reported anatomical
peculiarities of root, stem and leaf of the Punarnava species. In the Pharmacopoeias details about the microscopical standards of only root are given, but in the present investigation detailed microscopic standards for root, stem and leaf of all four species of Punarnava have been worked out. Deshmukh et al. (1957) reported zig-zag medullary rays and abnormal secondary growth in B. diffusa L. root while in the present investigation root did not show zigzag medullary rays. Singh and Udupa (1972 a) reported indistinct endodermis, 1-2 layered pericycle, xylem vessels with five or more radial groups of vessels and crescent shaped patches of phloem for the root of B. diffusa L. However, vessels in groups of 2-8 in the radial rows are reported in Ayurvedic Pharmacopoeia (2001). In the Pharmacopoeias, details of cork region of the root of B. diffusa L. are not given, however, these are recorded in the present investigation. The cork and cortex is recorded many layered in B. diffusa L. and B. repanda Willd. while in B. erecta L. and T. portulacastrum L. it is few layered. In T. portulacastrum L. the vessels are in radial rows while in other species these are in group.

Surange et al. (1977) reported cork cells, phellogen, cortex, distinct pericycle and abnormal secondary growth in the stem of B. chinensis (Burm.f.) Druce. while the stem of B. repanda Willd. distinct pericycle and abnormal secondary growth is recorded. Xylem, vascular bundle number, phloem arrangement and number of vascular bundle arrangement found characteristic in Punarnava species.

The anatomical characteristics observed in the leaf of T. portulacastrum L. are quite comparable with the characteristics
recorded by Datta and Mukerji (1952) for the leaf of *T. portulacastrum* L. except the size and number of layers. The presence of large epidermal cells (upper) at the intervals in the lamina is a distinct unique feature of *T. portulacastrum* L. leaf among Punarnava species.

The structural peculiarities of the tissue elements viz. vessels, fibres, tracheids, ray parenchyma cells, etc. were studied by maceration studies or elemental analysis. In the present investigation the vessels with simple pits reticulate thickenings and 140 X 74.5 μ dimensions are recorded in *B. diffusa* L. The thick walled tracheids and aseptate, elongated fibres with pointed end were also observed in *B. diffusa* L. Similar observations have been reported for *B. diffusa* L. root in Ayurvedic Pharmacopoeia (2001). In *B. erecta* L. reticulate or scalariform thickenings are observed. In present investigation large sized 195.7μ (110.0-260.0 μ) X 22.0μ (16.5-43.0μ) tracheids in *B. diffusa* L. root and small sized 130.2μ (52.0-170.0 μ) X 18.2 μ (10.0-19.5 μ) tracheids recorded in *B. repanda* Willd. The structural differences and variations in the dimensions in tissue elements serve as identification mark for the closely related species.

The quantitative microscopy study is included as one of the standards in the Pharmacopoeias. Paliwal (1980), Kannabiran (1973 a) and Surange *et al.* (1977) have carried out quantitative microscopy studies for Punarnava species. In Ayurvedic Pharmacopoeia (2001) quantitative standards for *B. diffusa* L. root are given. In the present investigation detailed quantitative microscopy study for starch grains, crystals and leaf constants have been carried out for all four Punarnava species.
The peculiarities of starch grains and calcium oxalate crystals serve as the diagnostic characters for the identification and differentiation of closely related species. Almost in all Pharmacopoeias the starch grain type and its size is mentioned. In *B. diffusa* L. simple and compound starch grains are having dimensions 8.35 μ (6.0-11.0 μ) X 8.46 μ (7.0-10.0 μ) observed while Anonymous (2001) has recorded the starch grains of 2.57-11.0 μ in diameter. The size of starch grains observed in the present investigations is quite comparable with the recorded size in Pharmacopoeias. Surange *et al.* (1973 a) reported that the starch grains of *Zaleya pentandra* L. (closely related genera to *T. portulacastrum* L.) are 8.36 X 7.6 μ in diameter while in present investigation, *T. portulacastrum* L. showed 10.12 X 9.87 μ diameter which is slightly higher than reported for *Z. pentandra* L.

It has also been observed that the starch grains of *B. repanda* Willd. are significantly larger in size, 11.8 μ (8.0-14.5 μ), as compared to other Punarnava species. Literature survey showed that the characteristics of starch grain especially the hilum and striations have not been reported so far. However, in the present investigation these characteristics showed significant differentiation among Punarnava species. In *B. diffusa* L. the hilum is seen as line while it is seen as 2-5 rayed fissure in *B. repanda* Willd. In the present investigation the length of crystal bundle is recorded 145.0 μ for *B. erecta* L. However, Kannabiran (1973 a) have recorded 155.0 μ (130.0-195.0 μ) length of crystal bundles in *B. punarnava* Saha & Krish. The observations recorded in the present investigation match with the observations recorded by Kannabiran, (1973 a).
In *B. repanda* Willd. 155.8 μ (72.2-178.2 μ) length of the bundle of crystal is recorded, however, Surange *et al.* (1977) recorded small sized 49.4-102.0 μ bundles of crystals in *B. chinensis* (Burm.f) Druce (= *B. repanda* Willd.). In *B. diffusa* L., *B. erecta* L. and *B. repanda* Willd. the needle shaped crystals are present. However, prismatic calcium oxalate crystals with 21.76 (11.0-40.0 μ) X 14.11 (8.0-35.0 μ) dimensions are recorded in *T. portulacastrum* L. The presence of prismatic crystals is a diagnostic feature of *T. portulacastrum* L. among the Punarnava species.

The leaf constants viz. stomata per sq. mm, stomatal index, vein-islets number, vein-termination number and palisade ratio of all the four Punarnava species along with their average, range and standard deviation have been recorded. These constants showed significant differences among Punarnava species. In the present investigation stomatal index (upper epidermis) 8.92-14.50 is recorded for *B. diffusa* L. and in Ayurvedic Pharmacopoeia (1989) it is given 11.0-16.0. In the present investigations vein-islets number and palisade ratio are recorded 8.0-16.0 and 2.0-4.0 respectively for *B. diffusa* L while in Ayurvedic Pharmacopoeia (1989) these are reported. 9.0-15.0 and 3.5-6.5 respectively. This shows that the stomatal index, vein-islets number is comparable to the recorded value however, the palisade ratio is significantly low as compared to the recorded value in the Pharmacopoeia. In general, it is seen that the quantitative microscopy values for leaf constants recorded for *B. repanda* Willd. are quite low as compared to other Punarnava species except palisade ratio.
The palisade ratio recorded for *T. portulacastrum* L. is 1.20-3.50 while it is 2.0-4.0 for *B. repanda* Willd. Surange *et al.* (1977) reported the stomatal index 7.9 (5.0-10.0) and 42.6 (32.0-56.0) for upper and lower epidermis; vein-islets number 14.9 (13.5-22.0) and palisade ratio 2.2 (2.0-2.5) for *B. chinensis* (Burm.f.) Druce. However, these values are quite high as compared to the observed values in *B. repanda* Willd. and only the value given for palisade ratio 2.0-4.0 is comparable. The leaf constants reported by Kannabiran (1973 a) for *B. punarnava* Saha & Krish. are stomatal index (of both surfaces), 13.6-15.3, 11.7-18.4; vein-islets number, 8.0-11.0 and palisade ratio, 6.0-9.0. However, in the present investigation these are 13.0-19.56, 12.35-17.20, 9.0-18.0, 3.0-7.0 respectively for *B. erecta* L. These values are comparable with the values recorded by Kannabiran (1973 a). On the basis of the leaf constants Punarnava species can be well differentiated.

Very few researchers have carried out the qualitative analysis of powders of Punarnava species. Deshmukh (1957) reported starch grains and crystals from the root powder of *B. diffusa* L. In the present investigation detailed analysis of root, stem and leaf powders of Punarnava species has been carried out. The powder analysis included the microscopic studies, microchemical tests and fluorescence analysis. The organoleptic analysis of root powders showed that this analysis is very subjective and should be used only as an additional parameter. *B. repanda* Willd. root powder showed abundant starch grains while *T. portulacastrum* L. root powder exhibited abundant prismatic crystals.
In general, the microscopic analysis of root powder shows quite distinct differentiation in all the four Punarnava species especially in the size of starch grains, dimensions of crystals, type of crystals and as well as the characteristics of vessels, parenchyma cells, cork cells, tracheids, fibres, etc. An artificial key has been prepared for the identification of root powders of Punarnava species. In the present investigation, the powders of root, stem and leaf were treated with various reagents which gave an idea about the phytochemicals present in the powders.

In the root powder alkaloids, fats, flavonoids, lignin, proteins, reducing sugars, starch and steroids and terpenes were detected while aminoacids, mucilage, and tannins could not be detected. However, glycosides were detected only in *B. diffusa* L. root. In stem powder very few phytochemicals viz. fats, lignin, starch and tannins were detected while alkaloids, aminoacids, flavonoids, glycosides, mucilage, reducing sugars, steroids and terpenes could not be detected. The leaf powders showed the presence of flavonoids, lignins, proteins, reducing sugars and starch. However, alkaloids, aminoacids, fats, glycosides, mucilage and tannins could be detected.

Fluorescence analysis is used for the quick identification of the powders. It has been observed that the fluorescence is characteristic for the root, stem and leaf powders. Surange and Pendse (1972), Surange *et al.* (1973 a), Kannabiran (l.c.) and Surange *et al.* (l.c) recorded fluorescence for the Punarnava species. Kannabiran (1973 a) recorded dull brown colour with nitric acid for *B. punarnava* Saha & Krish. leaf powder however, in the present investigation *B. erecta* L.
leaf powder showed chestnut colour at 254 nm which is quite comparable. Surange et al. (1977) observed blue fluorescence with 1N NaOH for *B. chinensis* (Burm.f.) Druce stem powder while the fluorescence of sepia colour at 254 nm in *B. repanda* Willd. does not match with the observations of Surange et al., (1977). In general, the fluorescence showed by root, stem and leaf powder is quite distinct. An artificial key for identification of parts of Punarnava species is formulated on fluorescence analysis of root, stem and leaf powders. The fluorescence analysis can also be used for the differentiation of root, stem and leaf powders of a particular Punarnava species.

However, fluorescence analysis is having limited application in the drug evaluation and it can be used as an additional parameter for the differentiation of closely related species.

Chemical investigations viz. proximate analysis, ash values, extractive values, histochemical, phytochemical and chromatographic studies are important in the standardization of the drugs. The proximate analysis of Punarnava species gives the rough idea about the contents present in the plant material. In the present investigation total carbohydrates recorded are 69.879 %, total proteins 6.820%, total fats 0.202%, total crude fibres 6.84% and total ash 14.909% for *B. diffusa* L. root. While *B. repanda* Willd. root shows high percentage, 77.60 of carbohydrates and *T. portulacastrum* L. root shows high percentage, 0.348% of fats. For *B. diffusa* L. root Deshmukh et al. (1957) recorded 65.29% carbohydrates and total ash 8.88%; these are slightly lower than recorded in the present investigations for *B. diffusa* L. root.
However, they have recorded higher percent of crude proteins 9.43% and crude fibres 15.04% than recorded in the present investigations.

Quantitative standards regarding the identity, purity and strength such as total ash, acid insoluble ash and extractive values are given in different Pharmacopoeias. In the present investigation for *B. diffusa* L. total ash 14.909 and acid insoluble ash 0.620 are recorded. While Surange *et al.* (1973 b) reported total ash value 12.5 and acid insoluble ash 3.98 for *B. diffusa* L. root. The acid insoluble ash value is quite low as compared to the recorded value by Surange *et al.* (l.c.).

In the different Pharmacopoeias different standards for ash values are given. According to Ayurvedic Pharmacopoeia (1989) total ash value should not be more than 15% and acid insoluble ash value not more than 6%. These values are quite comparable with the obtained values in the present investigation. In Herbal Pharmacopoeia (1998) it is recorded that the total ash should not be more than 13% and acid insoluble ash should be not more than 4%. The total ash value recorded in present investigation is higher than the mentioned ash value in Herbal Pharmacopoeia (1998). However, the acid insoluble ash value is quite comparable with the reported value. In Ayurvedic Pharmacopoeia (2001) it is reported that the total ash value should not be more than 10% and acid insoluble ash value should not be more than 0.8%. The total ash value, 14.909 recorded in the present investigation does not match with it, however, the acid insoluble ash value is quite comparable. Surange and Pendse (l.c.) reported the total ash value 13.12 and acid insoluble ash value 4.2 for *B. repanda* Willd. root.
However, the observed total ash value for *B. repanda* Willd. root is 10.402 and acid insoluble ash value 2.20. These values are slightly lower as compared to the values reported by Surange and Pendse (1972).

For *Zaleya pentandra* (L.) Jeffrey root, the total ash value and acid insoluble ash value reported is 10.52 and 2.54 respectively (Surange *et al.* 1973 a) however, in the present investigation total ash value is 14.760 and acid insoluble ash value is 1.0 for *T. portulacastrum* L. The total ash value is quite high while the acid insoluble ash value is low that the reported ash values. The ash values are also recorded for the aerial parts viz. stem and leaf. The total ash value reported for stem is 9.184 and for leaf 17.230 of *B. diffusa* L. while Subramanian and Ramkrishnan (1965) reported total ash, 11.8% for the aerial parts of *B. diffusa* L. which is higher than reported total ash value for stem and lower than reported for leaf of *B. diffusa* L. However, in *B. punarnava* Saha & Krish. total ash value reported for stem is 9.6% while in the present investigation it is recorded 10.20 for *B. erecta* L. stem. The total ash value reported for *B. chinensis* (Burm.f.) Druce. for stem is 12.8 and for leaf is 22.11 (Surange *et al.*, 1977); however, in the present study it is observed 10.833 for stem and 20.093 for leaf for *B. repanda* Willd. It seems that these values are comparable.

Surange *et al.* (1973 b) have reported ethanol extractive value, 1.20 for *B. diffusa* L. root. It is quite comparable with the observed value, 1.17% for ethanol in the present investigation. In Ayurvedic Pharmacopoeia (2001) the reported ethanol extractive value is 4.0% for *B. diffusa* L. root which is quite high as compared with the observed
value 1.170%. Singh and Udupa (1972 b) reported petroleum ether extractive 0.82% for *B. diffusa* L. (whole plant) while it is observed 0.260%, 0.267% and 0.744% for root, stem and leaf respectively in the present investigation. Surange *et al.* (1973 b) reported 0.92% petroleum ether extractive value in *B. diffusa* L. root while it is recorded quite low 0.260% in the present investigation. In Ayurvedic Pharmacopoeia (2001) it is mentioned that the water extractive value for *B. diffusa* L. root should not be less than 10%, however, the present investigation it is reported 9.840%. Surange and Pendse (1972) reported extractive values 19.15, 4.0 and 0.95 respectively for water, alcohol and petroleum ether in *B. repanda* Willd. however, the recorded values in the present investigation for *B. repanda* Willd. are quite low, 8.175, 1.670 and 0.225 respectively for water, alcohol and petroleum ether. In the present investigation the extractive values recorded for stem and leaf recorded are 11.5, 19.5 for water; 4.180, 3.790 for ethanol and 0.287, 0.565 for petroleum ether extract respectively. These values are low as compared to the recorded extractive values by Surange *et al.* (1977) for *B. chinensis* (Burm.f.) Druce. They reported 15.35, 49.5 for water; 3.65, 13.4 for ethanol; 0.59, 2.4 for petroleum ether respectively for stem and leaf of *B. chinensis* (Burm.f.) Druce. The variation in the extractive values is mostly occurs may be due to change in the climatic conditions, soils conditions and collection period of samples (seasonal variations). In the present investigation the colour of extracts at ordinary light and under UV at 254 nm and 366 nm are also recorded. However, Chopra *et al.*, (1940) reported colour of the extracts at ordinary light only.
The extracts of Punarnava species showed distinct differentiation at ordinary light and under UV.

The activity of the particular drug is due to the presence of a particular phytochemical in appropriate concentration. In view of this an extensive histochemical, phytochemical and TLC studies have been carried out to detect the presence of various chemicals. The number of research workers (Misra and Tiwari, 1971; Surange et al., 1973 b; Patil 1977; Verma and Awasthi, 1979; Suri et al., 1982; Anonymous, 1989; Fadeyi et al., 1989; Anonymous, 1998 and Anonymous, 2001) have reported a variety of phytochemicals, viz. sugars, glycosides, sterols, flavones, flavonoids, phenolic compounds, alkaloids, tannins, glycosides (Punarnavoside) and glycoproteins in *B. diffusa* L. root. However, anthraquinones and saponins have not been detected in Punarnava species (Fadeyi, et al., 1989). The review presented by Srivastava et al., (1998) also revealed that a variety of chemicals viz. alkaloids (Purnarnavine), flavonoids (C-methyl flavone, Boeravinone A, B and C), lignans (liriodendrin and syringaresinol-mono-β-D-glucoside) phenolic glucosides (Punarnavoside), glycoprotein, steroids and triterpenoids (β-sitosterol), etc are present in Punarnava.

The literature survey showed that almost no histochemical studies has been carried out except the preliminary work of Deshmukh et al. (1957) in *B. diffusa* L. root. The detailed histochemical studies for root, stem and leaf of the Punarnava species has been carried out in the present investigation. The results of histochemical studies for root, stem and leaf showed that cellulose, lignin, proteins and starch were present in
root, stem and leaf. However, chitin, mucilage and saponins were not detected in root, stem and leaf. Alkaloids, steroids and terpenes were detected only in root and tannins in stem of Punarnava species. Glycosides could be detected only in roots of *B. diffusa* L. Aminoacids could be detected in leaf of Punarnava species.

After performing the histochemical tests, phytochemical tests were performed with alcohol, water and petroleum ether extracts. In root extracts alkaloids, carbohydrates, lipids, flavonoids, pentoses, proteins, reducing sugars, starch and steroids were detected, while, aminoacids, mucilage, saponins and tannins could not be detected, in root of Punarnava species. Anthraquinones and glycosides were detected only in roots of *B. diffusa* L. Surange *et al.* (1972) recorded negative tests for starch and tannins while positive tests for proteins, reducing sugars and alkaloids in *B. repanda* Willd. root. However, in the present investigation the positive test for starch, proteins, reducing sugars and alkaloids and negative test for tannins are recorded. Fadeyi *et al.*, (1989) could not detect anthraquinones but this was detected in *B. diffusa* L. root.

In stem extracts carbohydrates, fats, proteins, starch and tannins were detected while other phytochemicals viz. alkaloids, aminoacids, anthraquinones, flavonoids, glycosides, mucilage, pentoses, proteins, reducing sugars, saponins and steroids could not be detected. The leaf extracts showed positive tests for alkaloids, aminoacids, carbohydrates, flavonoids, pentoses, proteins, reducing sugars, starch and steroids while, negative tests for anthraquinones, mucilage, saponins and
tannins. Glycosides were detected in *B. diffusa* L. while fats only in *T. portulacastrum* L.

Surange *et al.* (1977) carried out phytochemical screening of the stem and leaf powder of *B. chinensis* (Burm.f.) Druce. They reported positive tests for proteins, sugars and alkaloids. However, in the present investigation the stem did not show positive test for alkaloids and sugars. This may be due to low concentration of these chemicals. Mehta *et al.* (1999) detected alkaloids, carbohydrates, steroids, phenolic compounds and tannins while negative tests are reported for fixed oils, fats, saponins and proteins in *T. portulacastrum* L. roots. However, in the present investigation tannins could not be detected in roots. It is seen that mucilage and saponins are absent in Punarnava species.

The histochemical studies and phytochemical tests showed that in stem the few phytochemicals are present as compared to the root and leaf.

Some of the chemicals which were detected in the histochemical and phytochemical tests were further analyzed with sensitive technique, Thin Layer Chromatography (TLC). TLC technique is mainly used for the detection, standardization, and determination of the ingredients, adulterants or substitutes for material under consideration. Munshi *et al.*, (1978) pointed out that TLC can be used for the standardization of Ayurvedic drugs. For the standardization of drugs, the TLC fingerprints need to be developed. So in the present investigation using extracts in ethanol and petroleum ether TLC fingerprints have been developed. The spots were visualized using ultraviolet (254 nm and 366 nm wave lengths), iodine, Dragendorff’s reagent and
1% Vanilline-50% phosphoric acid to detect the complete array of chemicals. The TLC pattern recorded for ethanol and petroleum ether extract with different visualizing reagents serves as the fingerprints of Punarnava species. In spite of the paramount importance of this technique, very few workers (Surange et al., 1973 b; Munshi et al., 1978; Chakraborti and Handa, 1989 a; Anonymous, 2001) have carried out TLC studies in Punarnava species. In Ayurvedic Pharmacopoeia (2001) the TLC standards for *B. diffusa* L. root are also included. Surange *et al.*, (1973 b) detected alkaloids of the genuine and market samples with help of Dragendorff’s reagent in chloroform : methanol (95:5) solvent system. However, this solvent system did not gave much resolution therefore, other solvent systems viz. chloroform: methanol (80:20) and methanol: chloroform: acetic acid: benzene (3:17:0.4:2) have been employed in the present investigation. The solvent system chloroform: methanol (80:20) showed 8, 5, 6, 4 number of spots respectively for *B. diffusa* L., *B. erecta* L., *B. repanda* Willd. and *T. portulacastrum* L. under UV (254 nm). While 7, 4, 4, 4 spots with 1% Vanilline-50% phosphoric acid. However, Dragendorff’s reagent (for the detection alkaloids) showed an orange coloured spots at Rf 0.56 and 0.95 in *B. diffusa* L. Surange *et al.* (1973 b) also detected steroids in petroleum ether extract in benzene: ethyl acetate (80:20) solvent system with 1% Vanilline-50% phosphoric acid. In the present investigations the steroids were also detected in petroleum ether extract in chloroform: methanol (80:20) solvent system. Anonymous (1998) recorded Rf 0.40 in methanol: chloroform: acetic acid: benzene
(3:17:0.4:2) solvent system after potassium permanganate spraying (to detect Punarnavoside). In the present investigation n-butanol fraction of *B. diffusa* L. showed one spot at Rf 0.41. This spot at Rf 0.41 was not detected in n-butanol fraction of the other Punarnava species. Thus indicating the presence of Punarnavoside only in *B. diffusa* L. The presence of alkaloids, steroids and Punarnavoside have been confirmed in roots of *B. diffusa* L. while in other species presence of steroids has been confirmed with TLC technique. The TLC fingerprints developed in this investigation can be used as a criterion for the identification for Punarnava species.

High Performance Thin Layer Chromatography (HPTLC) is a versatile analytical technique which is highly reproducible, require less amount of sample, has high separation efficiency and detects smallest possible contents. It is mainly used in determining content, uniformity, and purity profile. In the standardization process the development of HPTLC-fingerprints is an essential step. Akade et al., (1995) pointed out that the application of fingerprinting technique using HPTLC can give high level of quality control. However, the review of literature showed that HPTLC studies on Punarnava species have not been reported. So in the present investigation HPTLC studies have been carried out mainly to develop the HPTLC-fingerprints for Punarnava species. HPTLC-fingerprints were developed using ethanol extract in solvent system, methanol: chloroform: acetic acid: benzene (3:17:0.4:2) at 285 nm wave length. The number of peaks, 9, 12, 6 and 3 were recorded respectively for *B. diffusa* L., *B. erecta* L., *B. repanda* Willd.
and *T. portulacastrum* L. ethanol extract at 285 nm wave length. In this system one common peak was observed at Rf 0.87 in all the four Punarnava species. *B. diffusa* L and *B. repanda* Willd. showed three common peaks at Rf 0.69, 0.79 and 0.87 while only two common peaks at Rf 0.35 and 0.87 were observed for *B. diffusa* L. and *T. portulacastrum* L. However, four peaks were common in *B. erecta* L. and *B. repanda* Willd. at Rf 0.05, 0.57, 0.63, 0.87. The four unique peaks were recorded for *B. diffusa* at Rf 0.27, 0.38, 0.55 and 0.66, while, *B. erecta* L. exhibited seven unique spots at Rf 0.07, 0.13, 0.17, 0.20, 0.30, 0.44 and 0.72. It is also seen that for *T. portulacastrum* L. extract shows 3 peaks at 285 nm. The HPTLC pattern obtained can be used as diagnostic tool for the identification of the particular species as well as for the detection of the genuine and adulterated material.

The column chromatography studies were out to isolate 'Punarnavoside' from the ethanol extract of *B. diffusa* L. root. The physical and chemical properties of the isolated compound were recorded. In the present investigation detailed study of the Punarnavoside has been carried out. In the present investigation the melting point (184.5°C) and IR (Nujol): 3400 (OH), 1700 (C=O), 1625, 1610, 1560, 1310, 1210, 1180, 1070, 920 and 720 cm⁻¹ is recorded for isolated compound; it matches with the earlier recorded melting point and IR-spectra for the Punarnavoside by Jain and Khanna (1989). They reported melting point (185-186°C) and IR (KBr): 3400-3350 (OH), 2900, 1735 (C=O), 1650, 1510, 1460, 1420, 1330, 1270, 1230; 1190, 1160, 1120, 1070, 920, 900 and 830 cm⁻¹ for Punarnavoside.
Seth et al. (1986) recorded maximum absorption at 285 nm for Punarnavoside and in the present investigation the isolated compound showed maximum at same absorption. Thus it is confirmed that the compound isolated from root of *B. diffusa* L. through column chromatography technique is Punarnavoside.

In the present investigation the quantitative estimation of Punarnavine and Punarnavoside was carried out. The total percentage of Punarnavine estimated was 0.0605% in *B. diffusa* L. root. However, Chopra et al. (1940) reported about 0.044 and Basu and Sharma (1941) have reported 0.05% percentage of total alkaloids from *B. diffusa* L. root. It seems that in the present investigation the total yield, 0.0605% of Punarnavine is high which may be due to use of titration method for estimation. However, Shrivastava and Padhya (1995) observed maximum 2% alkaloid content in the roots of *in vitro* plants. This may be due to the more amount of extraction of alkaloids in the *in vitro* plants than the normal root powder. SukhDev (1967) pointed out that the ethanol and petroleum ether extracts of *B. diffusa* L. root give negative tests for alkaloids to which the diuretic property was first attributed. He reported that the ethanol extract of root contains a nitrogenous substance C<sub>5</sub>H<sub>9</sub>N<sub>2</sub>O<sub>6</sub> m.p. 225-232-235° C, which not responded to alkaloidal tests. However, in the present investigation Punarnava species showed positive tests for alkaloids.

Seth et al. (1986) and Anonymous (1998) estimated 0.03-0.05% yield of 'Punarnavoside' from *B. diffusa* L. root. The estimation of this compound was done according the procedure described by...
Jain and Khanna (1989). However, in the present investigation the maximum percentage yield 0.0213% as low as compared to the previously recorded values of Punarnavoside. This difference might be due to different collection conditions like season and area of collection. The estimation of Punarnavoside has been carried out only in *B. diffusa* L. as Punarnavoside could not be detected in other species.

Now-a-days the study of seasonal variations has become important because it may help to find the stage (or season) of plant at which it has maximum therapeutic value. The work of Chadramouli (1976) clearly points out that the seasonal variation studies are essential to find out the exact stage of growth and the time of collection. It has also been observed that the specificity and extent of the therapeutic action depends upon the nature and quantity of specific chemical constituents present in the drug. There is a relation in the particular season and the chemical contents so it is necessary to carry out the collection of the plants in a particular season. However, literature survey revealed that the seasonal variation studies have not been carried out in Punarnava species except some sporadic records (Srivastava *et al.*, 1998). In view of this, the seasonal variation studies have been carried out. For the assessment of the seasonal variation studies, physical constants viz. total ash contents, water-soluble ash and acid insoluble ash, alcohol and petroleum ether extractive values as well as the HPTLC pattern for Punarnava species (ethanol extract) were done. For the HPTLC studies the solvent system chloroform :methanol (80:20) was used and the peaks were visualized with UV (254 nm).
For *B. diffusa* L., minimum total ash value 11.820, water soluble ash value 7.650 and acid insoluble ash value, 1.265 maximum ethanol extractive 2.028, petroleum ether extractive 0.276 and highest number of peaks 7 (Rf 0.08-0.88) were observed for summer season. While *B. erecta* L. minimum, total ash value 9.410, water soluble ash value 5.612, acid insoluble ash value 0.765, maximum ethanol extractive 2.776, petroleum ether extractive 0.194 and highest number of peaks 8 (Rf 0.07-0.84) were observed for winter season. For *B. repanda* Willd. the minimum total ash value 9.362; water soluble ash value 7.100, acid insoluble ash value 1.320, maximum ethanol extractive 1.870, petroleum ether extractive 0.276 and highest number of peaks 4 (Rf 0.56-0.84) were recorded for summer season. The same number of peaks, four were also observed in the material collected in summer season. For *T. portulacastrum* L. minimum total ash value 9.455, water soluble ash value 4.383, acid insoluble ash value 1.168, maximum ethanol extractive 1.720, petroleum ether extractive 0.119 and highest number of peaks 2 (Rf 0.17-0.73) were observed for winter.

Srivastava *et al.* (1998) recorded that Punarnava has maximum antiinflammatory activity from the samples collected in the rainy season. In the present investigation the minimum spots have been recorded in the monsoon season for *B. diffusa* L.

The biological investigations are mainly carried out to study the potency of the particular crude drug or its isolated fractions(s). From the literature survey it was realized that Punarnava is one of the important drug in the folk medicine in the indigenous systems of medicine.
and in modern medicine. Various pharmacological properties and activities of Punarnava have been claimed. Native population has employed Punarnava since ancient period as folk medicine. Various folk or ethnobotanical uses of Punarnava has been reported by different research workers (Rajwar, 1983; Bhatt and Sabnis 1987; Godbole, 1993 and Vishwanathan and Singh, 1996). They have reported the uses of Punarnava for blood dysentery, corns, debility, chronic fever, curing of mouth pustules, healing of wounds, joint complaints, etc.

The research workers (Haravey, 1966; Bhalla et al., 1971; Gaitonde et al., 1974; Singh et al.,1974; Garg, 1976; Mungantiwar, et al., 1997 a; Patil, 1977; Verma and Awasthi, 1979; Awasthi and Mukherji, 1980; Ojewole and Adesina, 1985; Awasthi et al., 1985; Chopra et al., 1988; Verma et al., 1985; Chakraborti and Handa, 1989 b; Jain and Khanna, 1989; Kadota et al., 1989; Gulati et al.,1991; Akah and Nwambie, 1993; Sohni et al., 1995 a; Kumar et al., 1997; Mungantiwar et al.,1997 a and Mehta et al. 1999) have attributed various properties viz. antiinflammatory, hepatoprotective, diuretic, adaptogenic, antifertility, anticonvulsant, antifibrinolytic, antistress, antibacterial, antiviral, etc.

In view of the claims about antiinflammatory activity in ancient literature (Trivedi, 1932; Bose, 1982) and in ethnobotanical work (Rajwar, 1983 and Guha Bakshi et al., 1999) of Punarnava and sporadic work on acute and chronic antiinflammatory activity (Bhalla et al., 1971, Mudgal, 1974; Patil, 1977; Gupta, 1978) the antiinflammatory activity is tested in animal system. The local and systemic antiinflammatory activity
has been worked out in the present investigation. The observations on local antiinflammatory (TPA-induced mouse ear-edema) activity showed 60.69% and 63.57% inhibition of inflammation respectively at 1 mg/kg and 2 mg/kg for *B. diffusa* L. extract. Thus showing a remarkable significant local activity at both doses. However, *B. erecta* L. extract showed 8.47% and 51.93% inhibition of inflammation respectively at 1 mg/ear, 2 mg/ear dose. *B. erecta* L. showing local antiinflammatory activity only at 2mg/ear dose. *B. repanda* Willd. showed 4.57%, 11.05% inhibition of inflammation at 1 mg/ear and 2mg/ear dose while *T. portulacastrum* L showed 5.51% and 3.30% inhibition of inflammation. Thus clearly showing that *B. repanda* Willd. and *T. portulacastrum* L. did not show any activity at both doses. It is seen that *B. diffusa* L. is having remarkable significant local antiinflammatory activity while *B. erecta* L. also shows significant antiinflammatory activity at high dose only.

Systemic inflammatory activity has been tested with alcoholic extracts of Punarnava species. The percentage inflammation along with its SEM (Standard Error Mean) and t-test has been presented in the present investigation. *B. diffusa* L. ethanol extracts (100 mg/kg) showed 26.49%, 43.94% and 62.10% inflammation respectively after 1 h, 2 h and 4 h treatment. *B. diffusa* L. showed activity even at 4 hours indicates that it has action against kinins and prostaglandins.

The work of Patil (1977) has recorded significant acute antiinflammatory activity for ethanol extract (50%) *B. diffusa* L. The ethanol extracts of *B. repanda* Willd. and *T. portulacastrum* L.
(100 mg/kg) showed 36.45% and 32.29% inflammation respectively. It is seen that *B. repanda* Willd. and *T. portulacastrum* L. are having antiinflammatory activity in early phase by blocking activity of histamine and 5 HT. The significant acute antiinflammatory activity has been reported in *B. diffusa* L. Gupta (1978) 50% inhibition with *T. portulacastrum* L. ethanol extract (100 mg/kg, i.v.) of whole plant. *B. erecta* L. showed 47.26%, 78.23%, 118.68% inflammation at 1 h, 2 h and 4 h respectively. It clearly showed that there is no significant activity for *B. erecta* L.

Thus only *B. diffusa* L. having antiinflammatory activity and this justifies the claims made earlier in the ancient literature as well as in the ethnobotanical investigations.

The literature survey revealed that studies have been carried out on antibacterial (Singh *et al.*, 1974; Kumar *et al.*, 1997) and antiviral (Verma and Awasthi, 1979; Awasthi and Mukerjee, 1980; Verma and Awasthi, 1980; Awasthi *et al.*, 1985; Verma *et al.*, 1985) activities. However, studies on antifungal activity of the Punarnava species have not been reported so far. In view of this fact, the antifungal activity has been carried out by the spore germination and food poison (inhibitory zone) techniques using fungi viz. *Aspergillus niger* V. Teigh., *Colletotrichum gleosporioides* Penz., and *Fusarium moniliforme* Scheld. The antifungal activity was observed at higher concentrations especially at 0.5%. *B. diffusa* L. extract (0.5%) showed 37.50 and 37.33 percent of spore germination inhibition respectively for *F. moniliforme* Scheld. and *C. gleosporioides* Penz. The extract (0.5%) of *B. erecta* L.
showed 35.83 and 43.0 percent inhibition of spore germination respectively for *A. niger* V. Teigh. and *F. moniliforme* Scheld. The extract (0.5%) *B. repanda* Willd. showed 36.0 percent inhibition of spore germination for *C. gleosporioides* Penz. The extract (0.5%) of *T. portulacastrum* L. showed 35.50, 35.17 and 43.67 percent inhibition of spore germination respectively for *A. niger* V. Teigh, *F. moniliforme* Scheld. and *C. gleosporioides* Penz. Broadly, it is seen that the extracts of Punarnava species have ability to inhibit the spore germination at higher concentration (0.5%) and at the low concentrations the extracts are less effective. It is also observed that *B. repanda* Willd. extract is less effective to inhibit spore germination. For further confirmations of the antifungal activity, the poisoned food technique was employed and it showed that *B. diffusa* L. extract has antifungal activity at 0.5% concentration against *F. moniliforme* Scheld. and *C. gleosporioides* Penz. However, no activity against *A. niger* V. Teigh. have been observed. From the above observations it is seen that the extracts of *B. diffusa* L., *B. erecta* L. and *T. portulacastrum* L. have antifungal activity at higher concentrations. These investigatory results indicating that Punarnava species have antifungal activity and *T. portulacastrum* L. is more potent antifungal.

Market sample study is an important exercise in the Pharmacognostic studies. Therefore, detailed market sample studies were carried out in the present investigation. Extensive market survey was carried out and many market samples are obtained from different vendors. In the present investigation two market samples were worked out in
The market samples were in the form of root pieces, which showed differences in colour, surface and also in thickness. The transverse section of market sample 1 showed vessels in group while in market sample 2 these were in rows and not in a group. The maceration studies of two market samples also showed marked difference in dimensions of different tissue elements. The analysis of starch grains and crystals showed that in market sample 1 the hilum of starch grain as a line while in market sample 2 it was 2-3 rayed fissure. In market sample 1 the crystals were observed in form of raphides while in market sample 2 these were in the prismatic form. The crystals served as a diagnostic feature for identification of market sample 1 and 2. The powder analysis with respect to organoleptic, microscopic and fluorescence characteristics showed marked difference in both the samples. The proximate analysis showed total carbohydrates 70.0%, 71.45%; proteins 6.95, 6.5%; fats 0.206, 0.350; crude fibres 6.8, 6.0 and total ash 14.594, 13.7 and other organic constituents 1.45%, 2.0% respectively for market sample 1 and 2. The foreign organic matter, a standard for the impurity was recorded 1.0% and 97.3% respectively for market sample 1 and 2. A marked difference was also noted in the water-soluble ash and acid insoluble ash values of the market samples. The water-soluble ash 11.10, 5.0 and acid insoluble ash 0.60 and 1.2 are recorded respectively in market sample 1 and 2. The difference is also observed in the petroleum ether and water extractive values of the market samples. Petroleum ether extractive 0.250%, 0.108%; ethanol extractive 1.170%, 1.0% and water extractive 9.75, 6.48 are noted respectively in market sample 1 and 2.
The microchemical studies of powder as well the histochemical and phytochemical studies showed that many phytochemicals are common in both market samples except glycosides. The glycosides are detected only in Market sample 1. TLC studies also showed marked difference in Rf values and the number of spots.

Chromatography studies for ethanol extracts in chloroform: methanol (80:20) system at 254 nm showed 9 spots for market sample 1, while, 5 spots for market sample 2.

Overall observations on these samples clearly indicated that the market sample 1 tallied with *B. diffusa* L. while market sample 2 with *T. portulacastrum* L.