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***Boerhaavia diffusa* Linn.: A Review on its Phytochemical and Pharmacological Profile**

Babita Agrawal, Sunanda Das and Archana Pandey

Department of Chemistry, C.M.P. Degree College, University of Allahabad, Allahabad-211002, U.P., India

Corresponding Author: Babita Agrawal, Department of Chemistry, C.M.P. Degree College, University of Allahabad, Allahabad-211002, U.P., India Tel: 91-9415634419, 91-532-2640211

ABSTRACT

Boerhaavia species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are rich sources of alkaloids, steroids and flavones. *Boerhaavia diffusa* Linn. (Punarnava) has attracted a lot of attention due to its prevalent uses in Ayurvedic system of medicine. It is widely used in jaundice, hepatitis, oedema, oligurea, anemia, inflammation, eye diseases etc. Pharmacologists and clinicians have investigated *B. diffusa* for all these activities. In some cases the findings support the existing clinical uses. It possesses hepatoprotective, diuretic, anti-inflammatory, anti-stress and immunomodulation, antifertility, antimicrobial, antiviral and insecticidal activities. In conclusion, *B. diffusa* Linn. contains biologically active compounds that may serve as candidates for new drugs in the treatment and prevention of human and livestock diseases.

Key words: *Boerhaavia diffusa*, phytochemical constituents, bioactivities, ethanomedicine, hepatoprotective

INTRODUCTION

Ayurveda is a native Indian healthcare system which is currently used by million of people in India, Nepal and Sri Lanka for their day-to-day healthcare needs (Cooper, 2008; Goyal *et al.*, 2011). Plants have great potential uses, especially as traditional medicine and pharmacopoeial drug. A large proportions of the world's population depend on traditional medicine because of the scarcity, high costs of orthodox medicine (Tagboto and Townson, 2001; Hudaib *et al.*, 2008) and unpleasant side effects (Dalirsani *et al.*, 2011). A number of natural products have been used as lead compounds because of specific activity and low toxicity (Sanda *et al.*, 2011). Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents (Pandey *et al.*, 2010; Evans, 2000; Oladunmoye *et al.*, 2009). Many plants contain a variety of phytochemicals (Agrawal *et al.*, 1993; Agrawal and Singh, 2010; Hemlata *et al.*, 1994; Pandey *et al.*, 1995, 1997; Pandey and Shukla, 2001) which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (Newman *et al.*, 2003; Gilani and Atta-ur-Rahman, 2005; Srivastava *et al.*, 2011).

It is very important to have sufficient knowledge regarding herbs not only because of their widespread uses but also because they have potential to cause toxic reactions or interact with other drugs.

Boerhaavia diffusa Linn. popularly known as 'Punarnava' is an important rejuvenative drug used in Ayurveda. It is widely distributed throughout India and flourishes during rainy seasons. The aerial parts then disappear but revive or sprout again next year (Sivarajan and Balchandran, 1985). Matured whole plant constitutes the drug in Indian Herbal Pharmacopoeia (Handa *et al.*, 1998).

The aims of present study were to review the chemical constituents of *Boerhaavia diffusa* Linn. and their biological activities and highlight their potentials as candidates for new drugs that may be of value in the treatment and preventions of human and livestock diseases.

General botanical description and properties of *Boerhaavia diffusa*: *Boerhaavia diffusa* commonly known as 'Punarnava' belongs to the family Nyctaginaceae. It is a diffusely branched pubescent or glabrous, prostrate herbs, abundantly occurring as a weed throughout India, up to an altitude of 2000 m in the Himalayas.

B. diffusa has single, thick deep penetrating tap root bearing few rootlets occasionally brown (Surange and Pendse, 1972a). Root is stout and fusiform with a woody root stock. Stems are creeping, many arising from root stock, swollen at the nodes and often purple.

Leaves are broadly ovate with slightly rounded or pointed apex and rounded base. The upper surface of the leaf is green but lower surface is white. The margin is entire and often pink at maturity, flowers are bracteolate umbels and deep pink in color. They usually contain 2-3 stamens but sometime only one stamens in each flower is also observed. The fruits are small 2.5 mm long, oval, oblong, pubescent, simple, achene, bluntly five ribbed with single seed (Handa *et al.*, 1998).

Microscopically the mature root of *B. diffusa* shows a complete ring of wood surrounding the ventral vascular region. The secondary concentric zone of wood tissue consists of a few vessels radially arranged and separated by parenchyma which formed the actual wood mass.

The stem shows a single layer of epidermis consisting of cuboidal cells. The epidermis consisting of cuboidal cells. The epidermis is covered from outside by cuticle and large number of glandular hairs. The cortex consists of 1-3 layers of collenchymas cells and several layers of parenchyma. The endodermis is not distinct. Two cells thick layer of pericycle surround the stele. The stele showed several vascular bundles, few bigger ones situated in the center and others forming a ring around the central bundles.

The lamina of the leaf of *B. diffusa* shows multicellular glandular hairs on the surface. The epidermis and the hairs of the leaf are covered with a continuous layer of cuticle. The epidermis at the upper side generally consists of cuboidal and lower side of the tubular cells. The lamina is transversed by several veins each surrounded by permanent bundle sheath (Singh and Udupa, 1972).

Ethanomedicinal uses of *B. diffusa* Linn.: *B. diffusa* has a long history of uses by indigenous and tribal people and in Ayurvedic or natural herbal medicines (Dhar *et al.*, 1968). *B. diffusa* root extract strengthens, tones and balances the liver (hepatotonic) (Rawat *et al.*, 1997). It shows hepatoprotective activity (Chandan *et al.*, 1991; Das and Agarwal, 2011).

The plant is used in epilepsy, pain in abdomen, dysentery, prolapsus ani, fistula ani and poison of scolopendrids (Jain and Tarafdar, 1970); in pneumonia (Saxena, 1986); Jaundice (Kumar, 1992; Singh and Ali, 1992; Singh, 1993; Anis and Iqbal, 1994; Sudhakar and Chetty, 1998); anaemia, (John, 1984; Basak, 1997); as blood purifier (Tripathi *et al.*, 1996); in enlargement of spleen (Rajwar, 1983); as stomachic, emetic, laxative, expectorant, diuretic (Jha *et al.*, 1997); astringent,

antiasthmatic, in abdominal pain cough (Das and Kant, 1988); as anti inflammatory (Kapur, 1993); tonic, in urinary troubles, ascites, uterine bleeding (Srivastava *et al.*, 1986) in liver complaints, heart diseases (Rana *et al.*, 1996) in dropsy, gonorrhoea, oedema, as diuretic (Singh and Aswal, 1992; Singh, 1993) in haemorrhoids (Singh *et al.*, 1996) anaemia, colic, thoracic haemorrhage, constipation, heart disease, oedema, as antidote to rat bite poison and in rabies (Thakur *et al.*, 1992) in urinary troubles (Maheshwari and Singh, 1984) as antiasthmatic, in anasarea, ascites (Banerjee and Banerjee, 1986) oedema, oligurea, as antidote to snake bite (Chandra *et al.*, 1989). The fruits are used as a diuretic. The seeds are used as expectorant. carminative, tonic, anthelmintic in lumbago (Tripathi *et al.*, 1996); jaundice and gonorrhoea (Mishra and Verma, 1995). The shoot is used in dysuria, oligurea as haematinic and uterine stimulant (Borthakur, 1996). The leaves are used in ophthalmic disease (John, 1984; Shah and Gopal, 1985, 1986); stomach disorder (Biswas and Ahmed, 1987); cataract (Reddy *et al.*, 1988; Balajirao *et al.*, 1995); as analgesic, antiasthmatic, blood purifier, in dropsy, gonorrhoea, jaundice and for hastening delivery (Raj and Patel, 1978); as laxative and digestant (Yoganarasimhan *et al.*, 1982; Sudhakar and Chetty, 1998); appetizer and alexiteric (Chaudhuri *et al.*, 1995); antihypertensive (Singh *et al.*, 1998); in rheumatism (Rao, 1981); hypotension (Jha and Varma, 1996); indigestion, abdominal pain, splenomegaly (Yoganarasimhan *et al.*, 1979, 1982); as diuretic, in jaundice, gonorrhoea; in urinary troubles (John, 1984); liver ailments (Gopal and Shah, 1985); to check post partum haemorrhage (Singh and Pandey, 1980); for wound healing (Sebastian and Bhandari, 1984); for itch and eczema (Saxena and Vyas, 1981).

Phytochemical constituents isolated from *B. diffusa* Linn.: Phytochemicals are natural bioactive compounds found in plants, including the medicinal plants, fruits, vegetables, flowers, leaves, roots and fibers and they act as a defense system against diseases or more accurately protect plants against diseases (Krishnaiah *et al.*, 2009). The therapeutic potentials, including antioxidant, antimicrobial and anti-carcinogenic properties of higher plants are due to the presence of secondary metabolites (Canigueral *et al.*, 2008; Kaur and Arora, 2009). The medicinal values of these plants lie in bioactive phytochemical constituents that produce definite physiological actions on the human and animal body. Some of the most important bioactive phytochemical constituents are the glycosides, alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils and phenolic compounds (Harbone, 1984; Edeoga *et al.*, 2005; Okwu, 2005).

The *B. diffusa* plant contains a large number of such compounds as flavonoids, rotanoids, alkaloids, steroids, triterpenoids (Kadota *et al.*, 1989; Lami *et al.*, 1990, 1991a; Jain and Khanna, 1989). These biologically active chemical substance known as secondary metabolites in medicinal plants, form the foundations of modern prescription drugs (Sofowora, 1993).

In recent decades, there are many reports on the use of medicinal plants. From the studies, it was discovered that the exact amount of active chemical constituent are frequently unknown. In general, one or two markers of pharmacologically active components in herbs and or herbal mixtures are currently employed for: (1) evaluating the quality and authenticity of herbal medicine; (2) identification of single herb or herbal mixtures and (3) assessing the quantitative herbal compositions of a herbal product. It was discovered that multiple constituents are usually responsible for the therapeutic affects of the plants.

These multiple constituents may act synergistically and could hardly be separated into active parts. Moreover, the herbal constituents may vary depending on the harvest seasons, plant origins,

drying processes and other factors (Walker, 2004). For example the roots of *B. diffusa* are used for the treatment of various hepatic disorders. The effect of seasons, thickness of roots and form of dose (either aqueous or powder) were studied for their hepato-protective action (Rawat *et al.*, 1997). The results showed that an aqueous extract (2 mL kg⁻¹) of roots of diameter 1-3 cm, collected in the month of May (summer) exhibited marked protection of a majority of serum parameters, viz., SGOT, SGPT, SACP, SALP but not GLDH and bilirubin, thereby suggesting the proper size and time of collection of *B. diffusa* roots for the most desirable results. The studies also showed that administration of aqueous form of drug (2 mL kg⁻¹) had more hepato-protective activity than the powder form.

In a preliminary screening, plant revealed presence of sterols (Singh and Udupa, 1972), β -sitosterol (Srivastava *et al.*, 1972; Desai *et al.*, 1973) and alkaloids (Garg *et al.*, 1980). Presence of steroids, sugars and alkaloids was also reported (Shukla, 1982).

It contains about 0.04% of alkaloid known as punarnavine (C₁₇H₂₂N₂O, mp 236-237°C) (Surange and Pendse, 1972b) and punarnavoside, an anti fibrinolytic agent. It also contains about 6% of potassium nitrate, an oily substance and ursolic acid (Kokate *et al.*, 2005). The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid.

Hentriacontane, β -sitosterol and ursolic acid along with glucose, fructose and sucrose were isolated from the root (Misra and Tiwari, 1971).

A new C-methyl flavones characterized as 5,7-dihydroxy-6-8-dimethoxy flavones was reported from root (Gupta and Ahmed, 1984) and designated as boerhavone (Ahmed and Yu, 1992).

Four new compounds, boerhavisterol, boerhadiffusene, diffusarotenoid and boerhavanasteryl benzoate and a known rotenoid, boerhavinone A were isolated from the root and their structures elucidated as : 9,10-seco-stigmast-5,8 (9)-dien-3 β -ol; 1-(2',6',6'-trimethylcyclohex-1'-enyl)-11-(3''-3''-dimethylcyclohexyl)-4,8-dimethyl-undec-1-ene; 4,9-dihydroxy-10-methyl-6a-dehydrorotenoid-6-pentanoate; 27-O-(4'-benzoyl- β -D-glucopyranosyl) 9 β -lanost-5-en-3-one and 6-methoxy-9,11-dihydroxy-10-methyl-6-a, 12a-dihydrorotenoid, respectively (Gupta and Ali, 1998). Chemical structures of all of the active constituents has been presented in Fig. 1.

Many rotanoids have been isolated from the roots of the plant (Kadota *et al.*, 1989; Lami *et al.*, 1990, 1991b). These include a series of boeravinones viz., boeravinone A, boeravinone B, boeravinone C, boeravinone D, boeravinone E and boeravinone F.

Punarnavoside, a phenolic glycoside is reportedly present in roots (Seth *et al.*, 1986). Punarnavoside was later characterized as 2-glucopyrano-4-hydroxy-5-(p-hydroxy phenyl) propionyl diphenylmethane (Jain and Khanna, 1989).

Bioactive eupalitin 3-O- β -D-galactopyranoside (5,4'-dihydroxy 6,7-dimethoxy-flavonal-3-O- β -D-galactopyranoside) and eupalitin isolated from the alcoholic extract of *B. diffusa* leaves (Pandey *et al.*, 2005) exhibited immunosuppressive properties.

Eupalitin 3-O- β -D-galactopyranoside (1>2)- β -D-glucopyranoside is isolated from ethanolic extract of roots of *B. diffusa* (Agrawal *et al.*, 2011).

Four new compounds were isolated from *Boerhaavia diffusa* namely (i) eupalitin 3-O- β -D-galactopyranosyl-(1''>2'')-O- β -D-galactopyranoside, (ii) 3,3',5-trihydroxy-7-methoxyflavone (iii) 4',7-dihydroxy-3'-methylflavone and (iv) 3,4-dimethoxyphenyl-1-O- β -D-apiofuranosyl-(1''>3'')-O- β -D-glucopyranoside (Maurya *et al.*, 2007).

A new dihydroisofuranoxanthone, methyl 3,10-dihydro-11-hydroxy-1-methoxy-4,6-dimethyl-10-oxo-1H-furo [3,4-b] xanthenes-3-carboxylate, designated as borhavine, has been isolated from the benzene extract of the roots of *Boerhaavia diffusa* L. (Ahmed and Yu, 1992).

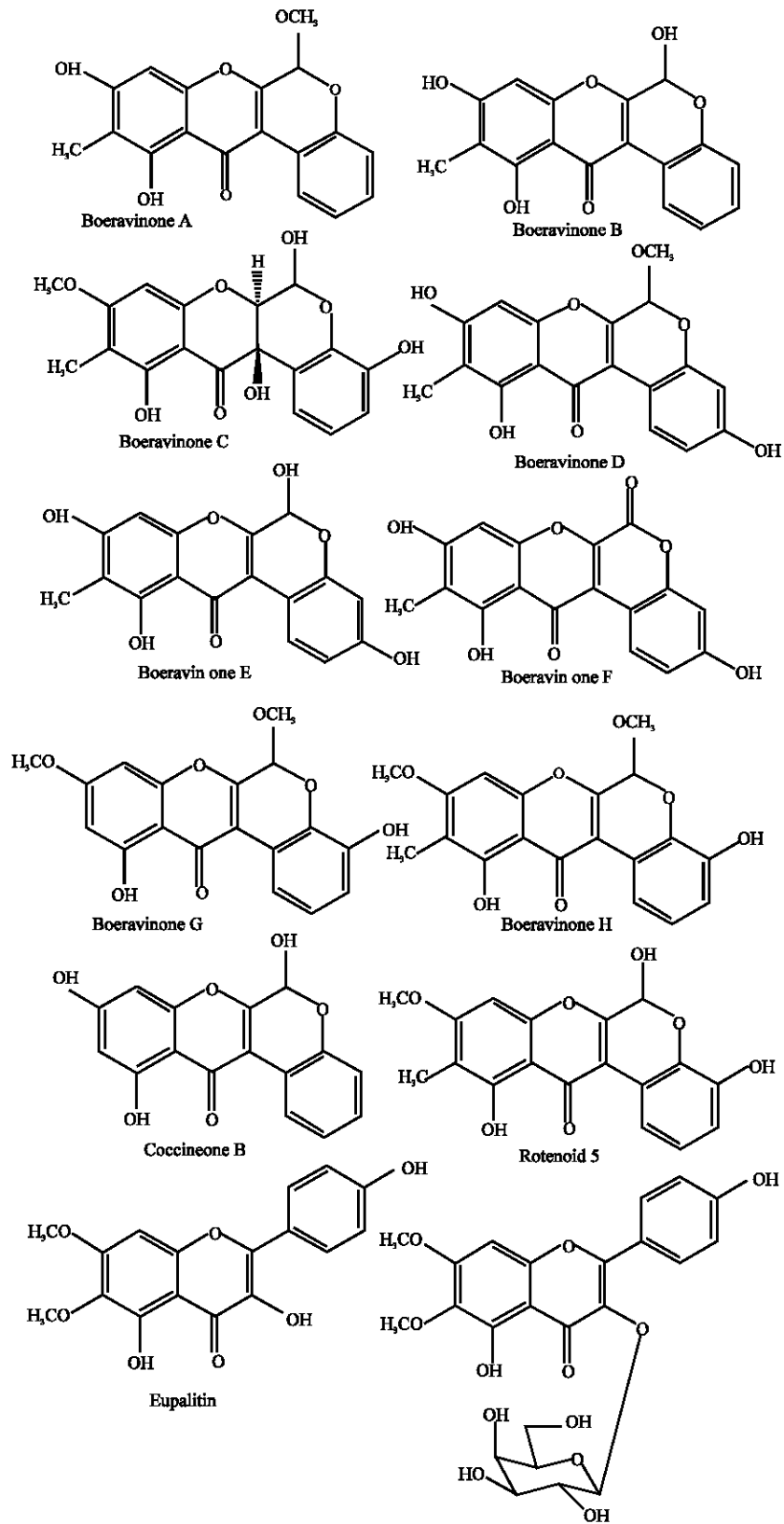


Fig. 1: Chemical structure of main constituents isolated from *Boerhaavia diffusa*

A metabolite profiling and biological study was undertaken in *Boerhaavia diffusa* leaves and roots and substantial differences were found between the two parts of the plant. The volatile composition was analysed for the first time using HS-SPME-GC-MS and several compounds including terpenes, phenyl propanoids, indole compounds, norisoprenoids, among others, were identified. Organic acid analysis was also performed allowing their characterization in this species for the first time and oxalic, ketoglutaric, pyruvic, quinic and fumaric acids were identified. Quantitative differences between two vegetal materials were found. Additionally, several flavonoids and one phenolic acid were also confirmed from the roots and leaves of the plant. These are quercetin 3-O-robinobioside, eupalitin 3-O-galactosyl (1→2) glucoside, Kaempferol 3-O-robinobioside, eupalitin 3-O-galactoside, quercetin and Caffeoyltartaric acid (Pereira *et al.*, 2009).

A bioassay guided separation of a methanolic extract obtained from the roots of *B. diffusa* L. allowed to isolate five compounds belonging to the class of rotenoids, out of which three compounds are known boeravinone D, boeravinone E and a rotenoid 5 along with two novel compounds boeravinone G and boeravinone H. Boeravinone G, boeravinone E and rotenoid 5 exhibited spasmolytic activity (Borrelli *et al.*, 2005).

Two known lignans viz., liriiodendrin and syringaresinol mono-β-D-glycoside have been isolated (Lami *et al.*, 1991a).

Two quinolizidine alkaloids identified as punarnavine-I and punarnavine-II were isolated from root, stem and leaves. The distribution of these alkaloids was maximum in stem and minimum in root. A relation was established between the growth process of the plant and biosynthesis of these two alkaloids. The alkaloidal content was initially low during commencement of pre-reproductive phase, gradually increased in different plant parts, becoming maximum during termination phase of reproductive stage (Nandi and Chatterjee, 1974).

Pharmacological and biological activities of *B. diffusa* Linn.: The plant has drawn lot of attention due to following biological activities:

Analgesic and anti-inflammatory activity: The analgesic property of aqueous extracts obtained from *B. diffusa*, mainly from the leaf juice of the plant. The data also confirmed the traditional indications. The mechanism underlay this analgesic effect remains unknown but the aqueous extract obtained from leaf juice is endowed with an apparently morphinomimetic central analgesic property (Hiruma-Lima *et al.*, 2000).

The aqueous and acetone extracts of the root, showed significant anti-inflammatory activity against carageenan-induced oedema and formaldehyde-induced arthritis in albino rats. The aqueous extract and an alkaloid significantly inhibited the increased serum amino transferase activity in arthritic animals similar to hydrocortisone. Liver ATP phosphohydrolase activity was also increased by aqueous extract and the alkaloid (Bhalla *et al.*, 1971).

The water insoluble alcoholic extract of different parts of the plant viz., root, stem, leaves and flowers and plant was studied for anti-inflammatory activity against carageenan-induced oedema in rats and for diuretic activity. The root and leaves were found to be most active (Mudgal, 1974) and the activity was found maximum during rainy season (Mudgal, 1975). No conclusive difference in diuretic and anti-inflammatory activities of leaves with or without flowers was observed (Mudgal *et al.*, 1977).

The effect of extract obtained from the root was studied on experimental acute pyelonephritis in rats. It reduced the inflammatory changes as well as the abscess formation in kidneys of animals infected with inoculation of *Escherichia coli*. It also reduced the bacterial count in the urine samples of infected animals (Singh *et al.*, 1988).

Hepatoprotective activity: The hydro alcoholic extract of roots of *B. diffusa* (HEBD) exhibited a significant protective action of liver evident by a reduction in elevated levels of serum lysosomal enzymes namely serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphate (ALP) in both CCl₄ and rifampicin-isonizid induced hepatotoxicity. Hence HEBD showed a dose dependent hepatoprotective activity (Desai *et al.*, 2008).

An alcoholic extract of whole plant of *B. diffusa* given orally exhibited hepatoprotective activity against experimentally induced carbon tetrachloride hepatotoxicity in rats and mice. The extract also produced an increase in normal bile flow in rats suggesting a strong choleric activity. The extract does not show any signs of toxicity upto an oral dose of 2 g kg⁻¹ in mice (Chandan *et al.*, 1991).

The effect of 50% ethanolic extract of roots of *B. diffusa* on Country Made Liquor (CML) induced hepatotoxicity was studied in albino rats. *B. diffusa* (100 mg/100 g body weight/day) protected the rats from hepatotoxic action of CML as evidenced by changes in serum alanine Aminotransferase (ALT), Triglycerides (TG), Cholesterol and total lipid levels in both serum and tissues. Histopathological studies showed marked reduction in fat deposits in animals receiving *B. diffusa* extract along with CML (Rajkumari *et al.*, 1991).

Different extracts and three isolates from extracts were subjected to *in vivo* and *in vitro* testing for antihepatotoxic activity. The petroleum ether, chloroform and methanol extracts and the total alkaloids (isolated from the methanolic extract) of the root were reported to slightly lower the increased SGOT, SGPT, SALP in rats treated with CCl₄. The chloroform and the methanol extracts exhibiting relatively better enzyme lowering effects were subjected to further fractionation yielding one of the isolates identified as rotenoid (6, 11-dihydroxy-9-10-dimethyl-4-methoxyrotenoid). The rotenoid was reported to lower the SGOT and SGPT at a dose of 100 µg mL⁻¹ of the media against CCl₄-intoxication on isolated rat hepatocytes. Similarly, the enzyme lowering action was observed with 500 mg kg⁻¹. i.p. administration of chloroform and methanolic extracts of the aerial parts. A steroid androst-5-ene analogue and a flavone, 6, 5'-dimethoxy-5, 7, 3'-trihydroxyflavone, isolated from the aerial parts, were also subjected to evaluation of antithepatotoxic activity against CCl₄-intoxication by *in vitro* method. The steroid significantly lowered SGPT and SGOT at a dose of 200 µg mL⁻¹ of the media, whereas the flavone significantly lowered the SGOT at a dose of 50 µg mL⁻¹ of the media (Chakraborti and Handa, 1989).

The effects of seasons, thickness of root and form of dose (either aqueous or powder) of root were studied for their hepatoprotective action in thioacetamide-induced liver toxicity in rats. The roots of different diameter were collected in three seasons, rainy, summer and winter. The results showed that an aqueous extract (2 mL kg⁻¹) of roots of diameter 1-3 cm, collected in the month of May, exhibited marked protection of majority of serum parameters viz. SGOT, SGPT, SACP and SALP but not GLDH and bilirubin, thereby suggesting the appropriate size and time of collection of root for better results. The studies also showed that administration of aqueous form of drug (2 mL kg⁻¹) had more hepatoprotective activity than the powder form. This was probably due to the better absorption of the liquid form through the intestinal tract (Rawat *et al.*, 1997).

Diuretic: The effect of extracts of red and white varieties of the plant was studied on diuresis and renal enzymes. Both the varieties exhibited diuretic activity in toads. Red variety showed comparatively less activity. It inhibited the activity of kidney's succinic dihydrogenase but showed stimulatory effect on it in lower doses. Inhibition produced by red variety was less than that of white variety. It depressed kidney tissue slice respiration but had no effect on kidney phosphatase. It stimulated the activity of kidney d-amino acid oxidase. The activity was more in white variety (Chowdhury and Sen, 1955).

In an experimental study, the aqueous extract of twigs in a dose of 2.5 mL 100 g⁻¹ in rats showed moderate diuretic activity, slightly more active than potassium acetate (Gujral *et al.*, 1955). The diuretic activity of water soluble fraction of root and leaves was reported in male albino rats and dogs. The activity was attributed to the presence of potassium content in it (Bhide *et al.*, 1958).

The petroleum ether extract of plant exhibited diuretic activity associated with increased sodium excretion in rats when given in a dose of 250 mg kg⁻¹ orally. The results were compared with polythiazide (Gaitonde *et al.*, 1974).

The glucosidic compound isolated from plant showed marked diuretic activity in saline-loaded rats with reference to urea as standard (Haravey, 1966).

Punarnava decoction showed good diuretic activity in rats in a dose of 1 mL 100 g⁻¹ with normal saline on alternate days for over 15 days period (Singh *et al.*, 1992).

The diuretic action of the plant was shown to be due to β -ecdysone isolated from the root of *B. diffusa* (Suri *et al.*, 1982).

Antistress and adaptogenic activity: The extract improved the stress tolerance by significantly increasing the swim duration and reducing the elevated WBC, blood glucose and plasma cortisol. Immunomodulatory activity was evaluated by carbon clearance and delayed hypersensitivity test. The extract significantly increased carbon clearance, indicating the stimulation of reticuloendothelial system. The extract also produced an increase in DTH response to SRBC in mice (Sumantha and Mustafa, 2007).

Immunomodulatory effect: The alkaloidal fraction from the roots of *Boerhaavia diffusa* was studied for its effect of cellular and humeral functions in mice. Orally administration is significantly inhibited SRBC-induced delayed hypersensitivity reactions in mice. However, the inhibition was observed only during post-immunisation drug treatment while no effect during pre-immunisation drug treatment, was observed. The study revealed that the alkaloidal fraction exhibited *in vivo* immunostimulatory activity without an *in vitro* effect in mice (Mungantiwar *et al.*, 1999).

In a study to evaluate the adaptogenic potential of root, the aqueous extract of the root powder was studied for its effect on *Escherichia coli*-induced abdominal sepsis, macrophage phagocytic activity in mice and on cold and forced swimming stress in rats. Pretreatment with root powder extract at a dose of 200 mg kg day⁻¹ orally for 15 days prior to *Escherichia coli* challenge produced significant leucocytosis with reduction in mortality in rats and also significantly increased macrophage phagocytic activity in mice. The plant extract reversed the stress-induced elevations in the levels of glucose, cholesterol, SGPT, BUN and reduction in triglycerides (Mungantiwar *et al.*, 1997).

The alkaloidal fraction isolated from the root was investigated for its effect on plasma cortisol, adrenal cortisol and humoral response in stressed rats. It exhibited restorative activity against stress-induced changes in plasma and adrenal cortisol levels. It also significantly augmented the antibody production in stressed rats as compared to control (Mungantiwar *et al.*, 1997).

Antifertility: The 50% aqueous ethanolic extract of root, when administered orally in experimental monkeys was found to stop intrauterine contraceptive device (IUCD)-induced bleeding at 50 mg kg⁻¹ bw (Seth *et al.*, 1986).

The ethanolic extract of root in a dose of 250 mg kg⁻¹ bw (daily) p.o. to pregnant albino female rats during the entire period of gestation did not show any teratogenic effects, as litter size and survival rate of fetuses were the same as for the normal control group and no foetal abnormality was detected (Singh *et al.*, 1991).

Immuno suppressive activity: *B. diffusa* hexane, chloroform and ethanol extracts and two pure compounds Bd-I (eupalitin-3-O-h-D-galactopyranoside) and Bd-II (eupalitin) were evaluated *in vitro* for their effect on T cell mitogen (phytohemagglutinin; PHA) stimulated proliferation of human Peripheral Blood Mononuclear Cell (PBMC), mixed lymphocyte culture, Lipopolysaccharide (LPS) stimulated nitric oxide production by RAW 264.7, PHA and LPS induced IL-2 and TNF- α production, in human PBMCs, superoxide production in neutrophils, human Natural Killer (NK) cell cytotoxicity and nuclear translocation of nuclear factor-k B and AP-1 in PHA stimulated PBMCs. The chloroform and ethanol extracts inhibited PHA stimulated two way MLR, NK cell cytotoxicity as well as LPS induced NO production by RAW 264.7; the hexane extract showed no activity. Bd-1 purified from the ethanolic extract at equivalent dose, inhibited PHA-stimulated proliferation of peripheral blood mononuclear cells, two-way MLR and NK cell cytotoxicity as well as LPS induced NO production by RAW 264.7 equally or more effectively than the parent ethanolic extract. Bd-I inhibited production of PHA stimulated IL-2 at the protein and mRNA transcript levels and LPS stimulated TNF- α production in human PBMCs; it also blocked the activation of DNA binding of nuclear factor-k B and AP-1, two major transcription factors centrally involved in expression of the IL-2 and IL-2R gene which are necessary for T cell activation and proliferation. Our results report selective immunosuppressive activity of *B. diffusa* leaf (Pandey *et al.*, 2005). A research is also carried out to evaluate the immunomodulatory properties of this plant extract on various *in vitro* tests such as human Natural Killer (NK) cell cytotoxicity, production of Nitric Oxide (NO) in mouse macrophage cells. RAW 264.7, interleukin-2 (IL-2), tumor necrosis factor- α (TNF- α), Intracytoplasmic Interferon- γ (IFN- γ) and expression of various cell surface markers on human Peripheral Blood Mononuclear Cells (PBMCs). Ethanolic extracts of *B. diffusa* roots inhibited human NK cell cytotoxicity *in vitro*, production of NO in mouse macrophage cells, IL-2 and TNF- α in human PBMCs. Intracytoplasmic IFN- γ and cell surface markers such, as CD16, CD25 and HLA-DR did not get affected on treatment with *B. diffusa* extract. Hence, it demonstrates immunosuppressive potential of ethanolic extract of *B. diffusa* (Mehrotra *et al.*, 2002a).

Antidiabetic activity: A study was carried out to investigate the effects of daily oral administration of aqueous solution of *Boerhaavia diffusa* L. leaf extract (BLEt) (200 mg kg⁻¹) for 4 weeks on blood glucose concentration and hepatic enzymes in normal and alloxan induced diabetic rats. A significant decrease in blood glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with BLEt (Pari and Satheesh, 2004). Chloroform extract of *B. diffusa* leaf produced dose dependent reduction in blood glucose in streptozotocin-induced NIDDM rats comparable to that of glibenelamide. The results indicate that the reduction in blood glucose produced by the extract is probably through rejuvenation of pancreatic beta-cells or through extra pancreatic action (Nalamolu *et al.*, 2004).

Radioprotective activity: In a study on the effect of the plant in radiation-induced haemopoietic injury in albino mice, pretreatment (in the dose of 260 g kg⁻¹ bw orally for 21 days) to mice exposed to total body irradiation (6 Gy) for 3 min showed significant increase in Hb and total RBC count. After irradiation, there was no fall in RBC count and Hb unlike in controls. The study indicated that the plant had selective effect on the erythroid compartment (Thali *et al.*, 1998).

Anti-metastatic activity: Administration of Punarnavine (40 mg kg⁻¹ body weight) prophylactically (95.25%), simultaneously (93.9%) and 10 days after tumor inoculation (80.1%) could inhibit the metastatic colony formation of melano main lungs. Survival rate of the metastatic tumor-bearing animals were increased significantly by the administration of Punarnavine in all the modalities compared to the metastasis bearing untreated control. These results correlated with the biochemical parameters such as lung collagen hydroxyproline, uronic acid, hexosamine, serum sialic acid, serum glutamyl transpeptidase and serum Vascular Endothelial Growth Factor (VEGF) levels and histopathological studies. Punarnavine administration could suppress or down regulate the expression of MMP-2, MMP-signal-regulated kinase) and VEGF in the lung tissue of metastasis-induced animals. Punarnavine could inhibit MMP-2 and MMP-9 protein expression in gelatin zymographic analysis of B16F-10 cells. These results indicate Punarnavine could inhibit the metastatic progression of B16F-10 melanoma cells in mice (Manu and Kuttan, 2009).

Prophylactic administration of the methanolic extract (0.5 mg dose⁻¹) inhibited the metastases formation by about 95% as compared to untreated control animals. There was 87% of inhibition in the lung metastases formation in syngeneic C57BL/6 mice, when the extract was administered simultaneously with tumour challenge (Leyon *et al.*, 2005). The total WBC count prior to irradiation was 7500±500 cells mm⁻³ which was reduced to 1500±500 cells mm⁻³ in the irradiated control group on day 9 after radiation exposure. But in th *B. diffusa* treated group, irradiated animals showed the lowest count on day 3 after irradiation (4000±400 cells mm⁻³). Where the count for irradiated control animals was 2100±440 cells mm⁻³. By day 9, the level reached 6250±470 cells mm⁻³ in *B. diffusa*-treated irradiated animals (Manu *et al.*, 2007).

Antioxidant activity: Leaves revealed stronger antioxidant activity than roots, the first analysis of volatile compounds of a widely used medicinal plant *B. diffusa*, using a HS-SPME-GC-MS technique directly into the headspace of the aqueous extract of the leaves and roots. In addition to phenolics (determined by HPLC-DAD), the organic acids (HPLC-UV) profile and *in vitro* antioxidant and anti acetylcholinesterase activities are described for the first time, providing further knowledge on this species chemistry and biological potential (Pereira *et al.*, 2009). Ethanol and methanol extracts were prepared and screened for *in-vitro* antioxidant activities using Ferric reducing power and Hydrogen peroxide scavenging activity. The activity was compared to standard antioxidant like ascorbic acid. Both the extract showed strong antioxidant activity in both the methods. Between these two extracts, ethanolic extract has shown better antioxidant activity as compared to methanolic extract in both the activities (Rachh *et al.*, 2009).

Antimicrobial activity: The methanol extract of *Boerhaavia diffusa* leaves had significant *in vitro* antimicrobial activity. Hence, further results revealed that among several pathogenic bacteria, only *Staphylococcus aureus* was susceptible for *Boerhaavia diffusa*. In *Boerhaavia diffusa*, maximum inhibition was observed in *Staphylococcus aureus* followed by *Bacillus megaterium* and *Bacillus cereus*, respectively at 50 µL concentration (Girish and Satish, 2008).

The alcoholic extract of root showed antimicrobial activity against *Staphylococcus aureus* whereas aqueous extract was active against *Escherichia coli* and inactive against *Staphylococcus aureus* (George *et al.*, 1947). The phosphate buffer and ether extracts of shoot showed antibiotic activity against *Staphylococcus aureus* and was inactive against *Escherichia coli* (Joshi and Magar, 1952). The alcoholic extract of the plant showed antibacterial activity against *Escherichia coli* *in vitro* studies (Singh *et al.*, 1974). The seed exhibited antibacterial activity against *Bacillus subtilis*, *Pseudomonas cichorii* and *Salmonella typhimurium* but was inactive against *Escherichia coli* (Sushil *et al.*, 1997). The aqueous extract of leaves of *B. erecta* and *B. diffusa* were screened for antibacterial activity against *Alkaligenes viscolactis*, *Aeromonas hydrophilla*, *Cytophaga* sp., *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Vib damsela*, *Bacillus cereus* and *Streptococcus pyogenes*. *B. diffusa* failed to exhibit antibacterial activity against all the bacteria but *B. erecta* was found to be active against *Alkaligenes viscolactis*, *Bacillus cereus* and *Streptococcus pyogenes* (Perumal Samy *et al.*, 1999).

The leaf extract did not exhibit antifungal activity against *Microsporium nanum* (Rai, 1987). The aqueous extract of root exhibited 21-50% inhibition of spore germination against *Curvularia lunata*, *Cylindrocarpon lichenicola*, *Fusarium solani* and *Myrothecium leucotrichum* (Gourinath and Manoharachary, 1991). The fresh leaf extract inhibited germ tube elongation of *Drechslera oryzae* (Ganeshan and Krishnaraju, 1995). The aqueous extract of leaves/stem/flowers/seed/plant exhibited antifungal activity against 3 keratinophilic fungi; moderate activity against *Microsporium gypseum* (39.01%), was less effective against *Chrysosporium tropicum* (25.63%) and showed minimum antimycotic activity against *Trichophyton terrestre* (1.76%) (Qureshi *et al.*, 1997).

Anti-viral activity: The *Boerhaavia diffusa* plant is reported to possess many pharmacological, clinical and antimicrobial properties. Recently it is observed potent antiviral efficacy of this plant against phytopathogenic viruses. The antiviral agent isolated from this plant was found to be a glycoprotein with a molecular weight of 16-20 kDa. Administered by foliar spraying in the field, this antiviral agent could protect some economically important crops against natural infection by plant viruses (Awasthi and Verma, 2006).

Maximum antiviral activity, in each case, was recorded with the aqueous extract of dried root powder applied before virus inoculation. The active principle was purified and isolated (Verma *et al.*, 1979). The roots of *B. diffusa* are a rich source of a basic protein which is used for inducing systemic resistance in many susceptible crops against commonly occurring viruses (Verma and Awasthi, 1979, 1980; Verma *et al.*, 1979; Awasthi *et al.*, 1984, 1985, 1989). This protein or antiviral agent was active against tobacco mosaic virus in *Nicotiana glutinosa*, *Datura metel*, *Chenopodium amaranticolor* and *Nicotiana tabacum* (Ky58 White Burlley and NP31); sunnhemp rosette virus in *Cyamopsis tetragonoloba*, *Vigna unguiculata* and *Crotalaria juncea*; and gomphrena mosaic virus in *Chenopodium amaranticolor*, *Vigna unguiculata* and *Gomphrena globosa* when applied a few hours (2-24 h) before inoculation by the respective inocula of viruses (Verma and Awasthi, 1979; Awasthi *et al.*, 1984). The antiviral agent was a basic glycoprotein (70-80% protein and 8-13% carbohydrates) with a molecular weight of 16-20 kDa as determined by gel filtration chromatography (Verma *et al.*, 1979). The resistance-inducing protein was found to be extremely thermostable (Verma and Awasthi, 1979). Following treatment with the systemic resistance inducing protein, the susceptible healthy host produces a virus inhibitory agent. The glycoprotein occurring in *B. diffusa* roots functions as a signal molecule and is of great interest as

it has a role in stimulating the defense systems of plants against viruses. Owing to the high antiviral efficacy of *B. diffusa* under laboratory conditions, it was tested under field conditions as well against a few viral diseases of economically important crops. The purified glycoprotein from *B. diffusa* reduced infection and multiplication of tomato yellow leaf curl virus papaya ring spot virus (Awasthi, 2000) and cucumber green mottle mosaic virus (Awasthi *et al.*, 2003). The aqueous crude extract from the dried roots was also found significantly active against a number of viruses mung bean yellow mosaic virus (Awasthi, 2000); bean common mosaic virus (Singh and Awasthi, 2002); bottle gourd mosaic virus in muskmelon (*Cucumis melo*), ridged gourd (*Luffa acutangula*) and bottle gourd (*Lagenaria siceraria*) (Awasthi and Kumar, 2003).

In treated plants, the antiviral agent from *B. diffusa* not only decreased disease symptom severity but also protected the plants against infection by viruses.

Root of *Boerhaavia diffusa* contains basal proteins which show high virus inhibitory activity against plant viruses. Root extract of this plant induce strong systemic resistance in susceptible host plant. In the study, we found that the BD-SRIP induces the resistances against the TMV infection (Lohani *et al.*, 2007).

The aqueous extract of the leaves inhibited potato virus Y infection on chilli plants (Suriachandraselvan and Narayanasamy, 1987).

Nitric oxide scavenging activity: The extracts of various polyherbal drugs exhibited dose-dependent NO scavenging activities and the potency was in the following order: *Abana* > *Chyavanaprasha* > *Triphala* > *Geriforte* > *Septilin* > *Mentat* > *Gingko biloba*. The present results suggest that the traditional Indian polyherbal crude drugs may be potent and novel therapeutic agents for scavenging of NO and thereby inhibit the pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite. These findings may also help to explain, at least in part, the pharmacological activities like rejuvenating adaptogenic, anti infection, anti-inflammatory, cardioprotective and neuroprotective activities of these traditional, clinically used non toxic drugs because NO is an important bioregulatory molecule which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial and antitumor activity (Jagetia *et al.*, 2004).

Adaptogen activity: Adaptogens seem to be useful during both adrenal hyper stress as well as adrenal hypo fatigue. By definition, an adaptogen implies the capability for bi directional or normalizing effects. The most important adaptogens for the adrenals include Panax Ginseng, Siberian Ginseng, Ashwagandha, Rhodiola, *Boerhaavia diffusa* and Holybasil Leaf Extract. *Boerhaavia diffusa* has the ability to support both adrenal over and under activation. In stressful conditions it has demonstrated the ability to buffer the elevations of serum cortisol and prevent the suppression of the immune system that takes place with elevated cortisol. On the other hand, *Boerhaavia diffusa* has also demonstrated the ability to improve cortisol levels with end stage adrenal exhaustion (Mungantiwar *et al.*, 1997).

Growth inhibition of struvite crystals: This *in vitro* study had been carried out in the presence of herbal extract of *Boerhaavia diffusa* Linn, by using single diffusion gel growth technique. Sodium metasilicate solution of specific gravity 1.05 and an aqueous solution of ammonium dihydrogen phosphate of 0.5 M concentration were mixed so that the pH value 7.0 could be set. After the gelatin, equal amount of supernatant solution of 1.0 M magnesium acetate prepared with

0.5 and 1% concentrations of the herbal extract of *B. diffusa* Linn. were gently poured on the set gels in the respective test tubes in the aseptic medium. The growth of crystals without and with herbal concentration of *B. diffusa* Linn. increased the inhibition of crystals also increased in the gel media as well as the dissolution of crystals at the gel-liquid interface increases. The de-fragmentation of some grown crystals was also noticed (Vaidya *et al.*, 2009).

Anti fibrinolytic activity: A study that evaluates the effect of anti-fibrinolytic Agents- Aminocapric Acid (-ACA). tranexamic acid (AMCA); anti-inflammatory drugs (indomethacin. ibuprofen. naproxen); and plant extract (root extract of *Boerhavia diffusa*) on endometrial histology of IUD-fitted menstruating monkeys. It is effective in reducing stromal edema, inflammation, and tortuosity of glands, and in increasing the degree of deposition of fibrin and platelets in the vessel lumen (Barthwal and Srivastava, 1990).

Chemopreventive action: In the present study, cancer chemo preventive property of *B. diffusa* was evaluated on 7,12-dimethyl Benz (a) anthracene (DMBA) induced skin papillomagnesis in male swiss albino mice (6-7 weeks old). This leads to the supposition that the inhibition of tumorigenesis by the plant extract might have been executed either by preventing the formation of active carcinogens from their precursors or by augmenting detoxification process, preventing promotional events in the mouse skin through free radical scavenging mechanism (Bharali *et al.*, 2003).

Genetic diversity analysis: *Boerhaavia diffusa* is extensively used in herbal medicines as well as in the Ayurvedic system, because it contains a set of clinically important compounds. In the present study, the genetic variability in *Boerhaavia diffusa* between accessions of different geographical origin within the Indian Territory is assessed through random amplified polymorphic DNA (RAPD) makers. Twenty-eight accessions of *Boerhaavia* were screened with eighteen primers of which nine were found to be the most informative. The degree of polymorphism was found to be high in accessions collected from different places of Uttar Pradesh (Set II) in comparison to other states of India (Set I). A relatively lower level of polymorphism was recorded in accessions collected from diverse locations around Lucknow (Set III). Accessions from neighboring geographical regions exhibited more similarity than those from distant regions (as revealed by the set I analysis). Certain diagnostic makers may be correlated with morphological character (s) such as plant type. BDL appeared most distinct and divergent from the rest of the accessions and the BDJ plant in set II also showed least similarity estimate. Fragments of 5.62 and 4.47 Kb with primer GN59 was found to be unique for BDP and BD2 having ovate leaf character. Whereas ovoid leaf genotype exhibited 0.79 Kb (GN34 primer) fragment. Similarly a unique band type (0.35 Kb) with primer GN83 was present in BDL and BDI that share light pink flower. Jaccard's and Nei and Li similarity coefficient values amongst the accession were in the range of 0.22 to 0.89 and 0.33 to 0.93, respectively Association of RAPD makers with the leaf characteristics, flower colour as well as with geographical locations has been made. This shows that RAPD makers are also useful for the study of genetic structure of *Boerhaavia* populations (Shukla *et al.*, 2003).

Bronchial asthma: Dried leaves are used in dhoomapana (smoking) in treatment of bronchial asthma. The leaf decoction is an excellent expectorant when decocted with punarnava (*Boerhaavia diffusa*) and then combined with ginger juice and black pepper (Kala *et al.*, 2009).

Antilymphoproliferative activity: It inhibited T cell mitogen phytohemagglutinin and concanavalin A-stimulated proliferation of human Peripheral Blood Mononuclear Cells (PBMC). It also inhibited purified protein derivative antigen-stimulated PBMC proliferation and human mixed lymphocyte culture. In addition, *B. diffusa* extract inhibited the growth of several cell lines of mouse and human origin, such as mouse macrophage cells (RAW 264.7) human macrophage cells (U937), human monocytic cells (THP-1), mouse fibroblast cells (L.929), human embryonic kidney cells (HEK293), mouse liver cells (BNLCL.2), African green monkey kidney cells (COS-1), mouse lymphoma cells (EL-4), human erythroleukemic cells (K562) and human T cells (Jurkat). (Mehrotra *et al.*, 2002b).

Antiproliferative and antiestrogenic activity: Treatment with varying concentrations of BME (20-320 $\mu\text{g mL}^{-1}$) resulted in moderate to very strong growth inhibition in MCF-7 cell lines. BME competed with [^3H]-estradiol for binding to ER with IC_{50} value of 320 25 g mL^{-1} . RT-PCR analysis revealed that BME reduced the mRNA expression of pS2 indicating the antiestrogenic action of BME. BME treatment for 48 h resulted in a remarkable increase in the number of MCF-7 cells in the G₀-G₁ fraction from 69.1 to 75.8% with a reciprocal decrease of cells in all other phases indicating cell cycle arrest at G₀-G₁ phase. Hence, it demonstrates that *Boerhaavia diffusa* possesses antiproliferative and antiestrogenic properties and suggest that it may have therapeutic potential in estrogen dependent breast cancers (Sreeja and Sreeja, 2009).

Insecticidal activity: Chemical investigation of the root resulted in isolation of insect moulting hormone which was structurally identified as-ecdysone. Butanol extract of root was bioassayed on housefly (*Musca domestica*) last instar larvae (Suri *et al.*, 1982). The hexane and acetone extracts of twigs showed insecticidal activity against *Culex p. fatigans* and *Musca domestica* (Deshmukh *et al.*, 1982).

Toxicity of *B. diffusa* Linn.: Vomiting may be associated with larger doses of *B. diffusa*.

CONCLUSION

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing bioactivities. In spite of the tremendous strides in modern medicine, numerous natural products from traditional medicinal plants have been introduced in the development of theoretical drugs. In addition, many products containing herbal extracts are sold in the Asian market as substitutes or supplements of modern medicine. Recent years, ethno-botanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use.

The objective of this review has been to show the recent advances in the exploration of plant *Boerhaavia diffusa* as phytotherapy and to illustrate its potential as a therapeutic agent. The available information in the literature on the bioactivities of the *Boerhaavia diffusa* shows that the plant contains compounds with strong pharmacological activities of potential clinical relevance and is a popular remedy among the various ethnic groups, Ayurvedic and traditional practitioners for treatment of various ailments. Researchers are exploring the therapeutic properties which are not known.

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