Boerhaavia diffusa: Bioactive Compounds and Pharmacological Activities

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Boerhaavia diffusa belonging to family Nyctaginaceae has a wide distribution, occurring on major part of the globe. It is known as Punarnava in Ayurveda and is a main ingredient in many formulations of Ayurveda. It is used as traditional medicine by indigenous people of many countries in the world for its protective role against inflammation, prostatic hyperplasia, diabetes, cancer, gastrointestinal problems, arthritis etc. The whole plant contains numerous bioactive compounds which are responsible for its pharmacological activities. Experiments are being done to evaluate full potential of the plant. The present review focuses on the bioactive compounds and pharmacological activities of B. diffusa. The study is carried out with the aim that it will be helpful for more research and wide acceptance of B. diffusa so that the plant which till now was used by indigenous people for its medicinal properties will become an ingredient of major mainstream medicines used to treat chronic human ailments.

Keywords: Boerhaavia diffusa, Bioactive compounds, Pharmacological activities, indigenous, antioxidants.

Boerhaavia diffusa is a species of flowering plants which belongs to family Nyctaginaceae (Four o’clock family). The genus ‘Boerhaavia’ is so named to honour Hermann Boerhaave who was a famous 18th century Dutch physician and the species is named ‘diffusa’ due to the typical diffuse branching of the plant (Mishra et al. 2014). It is also known as spreading Hogweed, Windflower, Red Spiderling, Tarvine, Satha, Punarnava, Itcit, Kathilla and by many other regional names. In ancient medicine, B. diffusa is commonly called as Punarnava because of its regeneration properties in rainy season. It is creeping perennial weed, prostrate herb, can be upto 1m long and with spreading branches. It occurs abundantly in ditches, marshy places and waste places during rains (Pooja et al. 2017). It is widely distributed in many countries located in tropical and subtropical regions like India, Sri Lanka, Egypt, Sudan, Ghana, South Africa, Nigeria, China, Australia, Philippines, Iran etc. (Chaudhary and Dantu 2018, Nayak and Thirunavoukkarasu 2016). Entire plant is used as vegetable at some places. In some parts of India like Assam and West Bengal the indigenous people cook leaves as a vegetable (Jana 2007).

Many bioactive compounds like tannins, flavonoids, alkaloids (punarnavine), glycosides, steroids, terpenoids, phenolic compounds, rotenoids (boeravinones A-O) etc. are reported in B. diffusa plants (Parmer et al. 2018, Shisode and Kareppa 2011, Krishnamoorthy et al. 2017). Due to its ethanopharmacological, chemical and therapeutic values, it is classified as rasayana in Ayurveda. Ayurvedic formulations such as Punarnavasava,
Sukumara ghrita, Punarnavadyarishtha, Punarnavadi mandura, Sothaghna Lepa, Maha Narayan Taila, Punarnavastaka kvatha curma, Punarnava guggulu, Punarnavadi kvatha curma and Varuni contain B. diffusa as main ingredient. These formulations are used to treat numerous ailments in humans like heart disease, sciatica, inflammation, diseases of abdomen, spleen and liver disorders, arthritis etc. (Mishra et al. 2014). It is used as ethnomedicine in other countries also like in Brazil it is used to treat hepatitis, renal disorders, liver disorders, edema etc; in Iran it is used to treat gonorrhea, joint pain, jaundice, intestinal gas etc; in Nigeria for epilepsy, asthma, fever etc; in Philippines as diuretic and purgative and in Ghana for asthma and boils (Nayak and Thirunavoukkarasu 2016).

Long term use of synthetic drugs for the treatment of various chronic diseases always creates some side effects. So there is need to move towards alternative drugs i.e. herbal drugs which have no side effects due to the presence of bioactive compounds (Pooja et al. 2017). But there is need to improve the acceptance of classical and ayurvedic medicines in the world market which can be done by advancements in ayurvedic research which should be evidence based (Chauhan et al. 2015). Phytochemicals need to be identified and quantified in plants important in traditional medicine. Dosage of the medicine should be determined by further research. This all would establish quality standards for efficacy and safety of the traditional medicines and they would get huge acceptance and market in the whole world. Due to wide occurrence and easy accessibility of B. diffusa, medicines prepared from it will be cheap and will be a boon for the health of people especially for the people in low and middle income countries. Researches done in the past and recent years relating to the analysis of phytochemicals and pharmacological activities in B. diffusa are thoroughly studied and analysed and presented in the present work in a comprehensive way.

**Bioactive compounds**

Phytochemical analysis of extracts prepared from B. diffusa roots by Shisode and Kareppa (2011), Parmer et al. (2018) and Pooja et al. (2017) has revealed the presence of flavonoids, phenolic compounds, saponins, glycosides, alkaloids, tannins, terpenoids and steroids. Ethylacetate, diethyl ether, ethanol, aqueous, chloroform and methanol extracts prepared from B. diffusa leaves were subjected to method of Harborne to test the presence of different phytochemicals by Umamaheswari et al. (2010). The qualitative analysis revealed the presence of different phytochemicals such as phenols, quinones, proteins, amino acids, saponins, carbohydrates, sterols, furanoids, alkaloids, glycosides, triterpenoids, flavonoids and tannins. The different extracts showed different constituency relating to the number of above phytochemicals. Three rotenoids, namely boeravinone A, B and C and 3-O-(6'-palmitoyl-â-D-glucopyranosyl) sitosterol, were isolated and their structure was determined by Lami et al. (1990) and Kadota et al. (1989). Boeravinone B was identified and quantified by employing HPLC in hydroalcoholic extracts made from whole plant of B. diffusa and also in its polyherbal formulation (Singh et al. 2017). The results showed that boeravinone B in the extract of B. diffusa was 0.041% w/w and in the polyherbal formulation was 0.011%. It scientifically validated the ratio of B. diffusa (ingredient) in the finished drug as one fourth of the finished product consisted of the ingredient and complied with the API limit. So the research established a criterion for global acceptance of ayurvedic drugs. Boeravinone B was isolated, purified and characterized from methanolic extracts of B. diffusa by Krishnamoorthy et al. (2017) by employing TLC and reverse phase HPLC. Aviello et al. (2011) first prepared methanol extracts of B. diffusa roots. After it with the help of Kupchan partitioning of the methanol extract four different fractions were obtained i.e. chloroform, n-hexane, n-butanol and carbon tetrachloride (CCl4). As the CCl4 fraction showed high antioxidant activity, it was subjected to further separation. Three rotenoids namely boeravinone D, boeravinone G and boeravinone H were isolated by using sequential silica gel column chromatography and HPLC. Their spectral data was compared with that reported in literature and so their structures were determined. Phenolic composition of B. diffusa collected from different locations was identified by Ferreres et al. (2005) by using HPLC-PAD-MS/MS. They identified ten phenolic compounds: quercetin-3-O- rhamnosyl(1-6)galactoside, kaempferol 3-O-(2"- rhamnosyl)-robinobioside, 3,4-dihydroxy-5- methoxycinnamoyl-rhamnoside, 3,5,4'-trihydroxy-
6,7-dimethoxyflavone 3-O-galactosyl(1'12) glucoside, quercetin 3-O-(2"-rhamnosyl)-robinobioside, kaempferol 3-O-robinobioside, caffeoyltartaric acid, quercetin, euphalitin 3-O-galactoside and kaempferol. The major phenolic compound in leaves was quercetin 3-O-(2"-rhamnosyl)-robinobioside and in roots was caffeoyltartaric acid. The results showed that the phenolic composition was influenced by the geographic origin of the plants. In that case also the nature of soil was the main responsible factor.

**Pharmacological activities**

**Cardioprotective effect**

Modern drugs used to treat various diseases always have some side effects. Its example can be seen in dual, healing as well as harming, effects of anthacycline drug, doxorubicin. It is an anticancer drug used to treat ovarian cancer, acute leukemia’s, breast cancer and Hodgkin and non-Hodgkin lymphoma (Kim et al. 2006). It kills cancerous cells by intercalating with DNA and inducing apoptosis of cells. It also results into generation of reactive oxygen species, to which particularly the anti-oxidant defenses of heart are susceptible. Scientists are making efforts to have some herbal remedies of the problem. *B. diffusa* has been traditionally used in Ayurveda to treat cardiac disorders (Kirtikar and Basu 1991). Studies were undertaken by Nimbal and Koti (2016) to investigate the preventive role of ethanolic extract made from *B. diffusa* whole plant against doxorubicin generated myocardial toxicity in rats. Administration of doxorubicin elevated the levels of biochemical parameters such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase; biomarker enzymes like creatine kinase and lactate dehydrogenase whereas reduced the levels of antioxidant enzymes such as catalase, glutathione and superoxide dismutase. The levels of all the above biochemical parameters, biomarker enzymes and antioxidant enzymes were normalized in the rats which were pretreated with the ethanolic extract of *B. diffusa*. So the data evidently proved the cardioprotective effect of *B. diffusa*, which was attributed to its antioxidant activity.

**Treatment of prostatic hyperplasia**

Benign prostatic hyperplasia (BPH) is common in elderly men as its prevalence is estimated to be 85%. Affected men have enlarged prostate gland and experience troubles during urination (Lee 2008). Investigation was carried out by Vyas et al. (2013) to study the effect of hydroalcoholic extracts prepared from roots of *B. diffusa* on BPH in rats. Testosterone dissolved in arachis oil was administered subcutaneously in male Wistar rats for 28 days to induce BPH in them. The model rats showed significant enhancement in prostate weight when compared with negative control rats. But the treatment with *B. diffusa* extract significantly reduced the prostate weight. Incubation with *B. diffusa* extract attenuated the contractile response of prostate gland and vas deferens which was elicited by exogenous application of noradrenaline. Histoarchitecture of prostate gland also showed improvements in rats treated with both testosterone and *B. diffusa* as compared to the model rats. It was concluded that anti-proliferative and anti-inflammatory properties of bioactive components in *B. diffusa* extracts might had relaxed prostatic smooth muscles and relieved the urinary symptoms of BPH disease.

**Anti-inflammatory action**

Inflammatory reactions are usually treated by taking nonsteroidal anti-inflammatory drugs. But the use of these preferred drugs causes unwanted side effects such as inhibiting the protective cyclooxygenase enzyme in gastric mucosa which results in gastrointestinal damage (Rang et al. 2008). So there is arising more interest in natural drugs which can treat inflammation without any side effects. Anti-inflammatory effect of *B. diffusa* was evaluated in rats by Sudhamadhuri and Kalasker (2014). Aqueous extracts were prepared from the plant leaves and their activity was determined on sub acute inflammation (cotton pellet induced granuloma) and acute inflammation (carrageenan induced paw edema) in rats. Pre-administration of the extracts (200 and 400 mg/kg) to the rats resulted in dose-dependent anti-inflammatory activity against sub-acute as well as acute inflammation. It was concluded that the above effects of the extracts were probably due to inhibition of chemical mediators of inflammation.

**Anxiolytic activity**

Anxiolytic activity of hydroalcoholic extract of *B. diffusa* leaves was evaluated in rats by Gadekar et al. (2011). Haloperidol Induced Catalepsy method, ketamine induced sleep, Elevated Plus Maze test and Hole Board Test were
used to study anxiolytic effect of the extract. The results revealed that hydroalcoholic extracts of *B. diffusa* had significant anxiolytic activity which was comparable to standard drug Diazepam (0.5 mg/kg).

**Protective effect on gastrointestinal problems**

The disruption of integrity of stomach mucosa results in peptic ulcer disease. It results from imbalance between factors responsible for gastroduodenal defense (bicarbonate secretions, mucosal blood flow, prostaglandins and mucus) and factors promoting mucosal damage (*Helicobacter pylori* infection, pepsin, gastric acid) (Devi and Jyothi 2015). The anti-ulcer activities of *B. diffusa* are contributed by several mechanisms such as its healing effects, regulation of gastric secretions, anti-inflammatory properties and mucus stimulatory effects (Scott and Ellis 1980). Healing effect of *B. diffusa* on gastric ulcers had been investigated by Devi and Jyothi (2015). Administration of root extract of *B. diffusa* alone and also along with Omeprazole efficiently resulted in reduction in development of gastric ulcer secretion in indomethacin induced gastric ulcers, pylorus ligation induced gastric ulcers and stress induced duodenal and gastric ulcers in rats. They concluded that the healing effect of root extract of *B. diffusa* was due to decrease in gastric secretion and also due to reduced gastric action.

**Anticancer activity**

Radiotherapy, surgery and chemotherapy methods used to treat cancer are highly effective but exert numerous side effects. Also the cancer cells gradually become resistant to the treatments. To circumvent it scientists are doing numerous efforts to find herbal solutions to cancer. Remya *et al.* (2018) carried out in vitro experiments to study cytotoxicity of decoction prepared from root of *B. diffusa* in MCF-7 (Michigan Cancer Foundation-7) breast cell line. During 48 hours of incubation in MCF-7 breast cell line, the test sample of *B. diffusa* at 800 µg/ml concentration showed cytotoxicity of about 65.1 ± 1.2. It proved the anticancer property of *B. diffusa* extracts. Anticancer activity of ethanol extract of *B. diffusa* leaves against Dalton’s ascitic lymphoma in mice is also reported by Kayande and Kushwah (2014).

**Antimicrobial activity**

Antibacterial activity of ethanolic extracts prepared from roots, stem and leaves of *B. diffusa* was evaluated by Majgaine and Verma (2017). Tests were conducted on three bacteria types *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. Reduction in colony diameter (zone of inhibition) was the criterion to measure anti-bacterial activity of the extracts. Roots extracts exhibited highest and stem extracts the least antibacterial activities. The extracts from the three parts of the plants were highest efficient against *S. typhi* followed by *S. aureus*. But no inhibitory effect of the extracts was noted against *E. coli*.

Umamaheshwari *et al.* (2010) qualitatively analyzed phytochemicals as well as antimicrobial functions of extracts of *B. diffusa* made in different solvents. The extracts were tested against gram positive bacteria like *Streptococcus faecalis*, *Bacillus subtilis*, *Micrococcus luteus* and *S. aureus* and also against gram negative bacteria like *Shigella flexneri*, *Proteus vulgaris*, *S. typhi*, *E. coli*, *Vibrio cholerae*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Inhibition zone diameter was calculated to determine the extract’s antibacterial activity against the test organisms. This property was attributed to the presence of phytochemicals such as flavonoids, sterols, phenols, alkaloids, tannins, quinones, saponins etc. It was noted that the extracts such as methanol extract, ethylacetate extract, chloroform extract, aqueous extract and ethanol extract showed antibacterial activity.

*B. diffusa* provided protection against pathogenic fungi and bacteria of humans was investigated by Wagh and Vidhale (2010). Decoction prepared from *B. diffusa* roots was effective against all the gram negative bacteria tested i.e. *S. typhi*, *P. vulgaris*, *E. coli*, *Enterobacter aerogenes*, *P. aeruginosa*, *Salmonella typhimurium* and *K. pneumoniae*. *Enterococcus faecalis* was the only gram positive bacterium which was sensitive to the decoction. Fungus, *Candida glabrata* showed high sensitivity to the decoction. The studies showed broad spectrum antimicrobial efficacy of *B. diffusa*.

In vitro experiments were performed by Ramachandra *et al.* (2012) to study antibacterial potential of *B. diffusa*. Chloroform, petroleum ether and methanol extracts were prepared from *B. diffusa* roots and aerial parts. The antibacterial efficacy of the extracts were tested against six bacteria types like *Agrobacterium tumefaciens*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. Reduction in colony diameter (zone of inhibition) was the criterion to measure anti-bacterial activity of the extracts. Roots extracts exhibited highest and stem extracts the least antibacterial activities. The extracts from the three parts of the plants were highest efficient against *S. typhi* followed by *S. aureus*. But no inhibitory effect of the extracts was noted against *E. coli*.

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B. subtilis, E. coli, P. aeruginosa, S. aureus and K. pneumoniae by using agar well plate method. In case of the extracts prepared from the aerial parts, the methanol extract had strong antibacterial property and the most susceptible bacteria were E. coli. Methanol extract prepared from the roots was most effective against S. aureus. So the experiments revealed that the whole plant of B. diffusa possess antibacterial properties.

**Protection against harmful radiations**

B. diffusa has the potential for protection against damage induced by exposure to radiations. To investigate it Manu et al. (2007) prepared hydroalcoholic extracts of B. diffusa and tested their effects using in vivo mice models. Mice were given whole body exposure of single acute dose of gamma radiation (600 rads) and were treated intraperitoneally with the extract (20 mg/kg). Estimation of intestinal glutathione, maturing monocytes and bone marrow cellularity indicated that the extract provided considerable protection to most affected tissues (intestine and bone marrow). The extract administration also normalized the total white blood cell count which was drastically lowered down by the radiation exposure. Levels of liver and serum alkaline phosphatase in mice were elevated by the radiation exposure. Their levels were reduced after treatment with B. diffusa extract. The irradiated animals also had increased lipid peroxidation level in serum as well as in liver, but B. diffusa extract administration significantly lowered down the lipid peroxidation level. All the results proved that whole plant of B. diffusa has potential for protection against radiation damage which may be attributed to improved immune status of irradiated mice after treatment with B. diffusa extract (Manu et al. 2007).

**Hepatoprotective activity**

Madagundi et al. (2016) isolated the endophyte Bacillus cereus from the roots of B. diffusa, prepared ethyl acetate and chloroform extracts from it and studied their hepatoprotective activities in rats. The extracts were studied for free radical scavenging activities. Out of the two, the ethyl acetate extract had significant IC$_{50}$ value and was used for further in vivo studies. Liver disorders were induced in rats by oral administration of CCl$_4$ (hepatotoxic chemical) to them. CCl$_4$ administration increased the levels of total and direct bilirubin, serum alkaline phosphatase, triglycerides, serum glutamic oxaloacetic transaminase and serum glutamate pyruvate transaminase in comparison to normal control. Treatment with the extracts of the endophytic bacteria reversed the elevated levels of all the above biochemicals. The extracts also maintained or increased the activity of enzymes like catalase and superoxide dismutase which play role in combating reactive oxygen species. All the results led to the conclusion that the extracts prepared from the endophytic bacteria of B. diffusa exhibit antioxidant and hepatoprotective functions.

Role of B. diffusa in curing hepatotoxicity induced by antituberculosis drug rifampicin in male albino Wistar rats was investigated by Muthulingam (2014). Tuberculosis is a major health problem and to treat it rifampicin therapy is given. Rifampicin cures tuberculosis but causes liver damage. To find a herbal cure for it Muthulingam (2014) first made methanolic extracts from leaves of B. diffusa. Hepatotoxicity was induced by administering rifampicin to the rats. It caused a substantial enhancement in the activities of aspartate aminotransferase, gamaglutamyl transpeptidase, bilirubin, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase whereas it decreased the amount of protein in serum as compared to the control. When the rats were given the methanolic extract of B. diffusa orally, rifampicin toxicity was alleviated as was evident from the nearly normalized levels of the above biochemicals. The extract having concentration 200 mg/kg body weight was the most effective one. So the experiments provide evidence that B. diffusa extracts can heal liver problems which was supposed to be due to the presence of flavonoids in the plant which have antihapatotoxic characteristics.

**Anti-arthritic activity**

Parmer et al. (2018) evaluated anti-arthritic effects of chloroform, water, methanol and petroleum ether extracts prepared from roots of B. diffusa in arthritic rats. Arthritis was induced in rats by injecting them with Freud’s Complete Adjuvant. Arthritic rats showed increased paw volume and decreased body weight. Arthritis also influenced hematological parameters such as increase in total WBC counts, increase in erythrocyte sedimentation rate and reduced RBCs and haemoglobin. Treatment of rats with the B. diffusa extracts decreased paw volume and increased body weight. Various
hematological parameters were also normalized like total WBC count became normal, erythrocyte sedimentation rate reduced and recovered RBCs and haemoglobin. Methanol extract gave the best results. Anti-arthritic activities of \textit{B. diffusa} root extracts were correlated with its flavonoids constituents as revealed by phytochemical analysis. 

**Anti-diabetic property**

Indigenous system of medicine in India has recommended many herbal medicines including \textit{B. diffusa} for curing diabetes (Madhumeha) (Nalamolu \textit{et al}. 2004). With the aim to prove and support the traditional usage of \textit{B. diffusa} for diabetes treatment, many research works have been done. Diabetic (Type I and Type II) patients with protein urea (\( \geq 500 \) mg/day), were studied by Singh \textit{et al}. (2011) for antiproteinuric effect of \textit{B. diffusa}. The patients were given \textit{B. diffusa} powder for six months. It was observed that there was some decrease in 24 hour urine protein in the patients after six months follow up.

Nalamolu \textit{et al}. (2004) performed experiments on diabetic rats. Non-insulin dependent diabetes mellitus was induced in rats by injecting streptozotocin. Chloroform extracts of \textit{B. diffusa} leaves were prepared and were orally administered (50, 100 and 200 mg/kg body weight) to rats daily for four weeks. Blood glucose was measured by glucose-oxidase method at weekly intervals for four weeks. Administration of the extracts of \textit{B. diffusa} lowered down the blood glucose levels in the diabetic rats in a dose dependent manner. The extract anti-diabetic activity was comparable to that of the anti-diabetic drug glibenclamide. Streptozotacin induces a massive damage to \( \beta \)-cells of islets of Langerhans and lower down the synthesis and release of insulin. Anti-diabetic action of the extract was attributed to its role in rejuvenation of pancreatic \( \beta \)-cells. Anti-diabetic activity of \textit{B. diffusa} has also been investigated by Chude \textit{et al}. (2001) and Pari and Satheesh (2004).

Antihyperglycaemic and renoprotective effects of ethanolic extracts of \textit{B. diffusa} were evaluated by Singh \textit{et al}. (2011) in diabetic rats. Diabetic animals showed reduced Na\(^+\)-K\(^-\) ATPase activity, altered ionic balance and glycemic dysregulation. Administration of \textit{B. diffusa} ethanolic extract maintained renal Na\(^+\)-K\(^-\) ATPase activity and ionic balance and resulted in significant reduction in diabetic hyperglycaemia. The effects were comparable to the effects of standard hypoglycaemic drug metformin.

**CONCLUSION**

The bioactive compounds like flavonoids, alkaloids, phenolic compounds, saponins, tannins, terpenoids, glycosides, steroids etc. are present in the extracts prepared from whole plant of \textit{B. diffusa} or its individual parts like leaves, stem and roots. These bioactive compounds are responsible for the healing effects of \textit{B. diffusa} against a number of human ailments like cancer, diabetes, inflammation, harmful radiations induced damage, gastrointestinal problems, microbial infections, prostatic hyperplasia, liver disorders, cardiac problems, anxiety problem etc. So, \textit{B. diffusa} which is used in traditional medicines, now its pharmacological potential is validated by a number of scientific experiments. But, further extensive research is needed to increase the acceptance and inclusion of \textit{B. diffusa} in mainstream medicines.

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