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A review of the plant *Boerhaavia diffusa*: its chemistry, pharmacology and therapeutical potential

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ABSTRACT

Introduction: *Boerhaavia diffusa*, a perennial creeping weed found in tropics and sub-tropics is a well known ethno-medicinal plant. The whole plant as well as its different parts (leaves, roots and stems) and plant extracts have been widely used in various traditional and folklore systems of medicine for treatment of various ailments. A number of phytochemicals e.g. flavonoids (C-methylflavone, 5,7-dihydroxy-3',4'-dimethoxy- 6,8-dimethylflavone, 3,5,4′- dihydroxy-6,7-dimethoxyflavone, 6', 5'-dimethoxy-5, 7, 3-trihydroxyflavone, borhavone, 3,3',5-trihydroxy-7-methoxyflavone, 4',7-dihydroxy-3'-methylflavone), alkaloids (punarnavine), glycosides (punarnavoside, eupalitin 3-O-β-D-galactopyranosyl-(1'''→2'')-O-β-D-galactopyranoside, 3,4-dimethoxyphenyl-1-O-β-D apiofuranosyl-(1''→3')-O-β-D-glucopyranoside), rotenoids (boeravinone A-H), steroids, triterpenoids, lipids, lignans, carbohydrates, proteins, and glycoproteins etc have been reported from the herb. Several researchers have confirmed biological, pharmacological and clinical activities of the plant and its phyto-constituents. Some of the promising effects of this plant include diuretic, hepatoprotective, anti-inflammatory, anti-fibrinolytic, anti-cancer, anti-diabetic, immuno-modulatory, immuno-suppressive, anti-lymphoproliferative, analgesic properties and used in treatment of pulmonary tuberculosis. Besides some less promising effects exhibited by this plant includes non-teratogenic, antioxidant, anti-viral activity against plant viruses, anti-bacterial, anti-fungal, adaptogenic, antiamoebic, lipotropic and anticonvulsant activity. An overview of chemical constituent of the plant, their pharmacological actions and clinical studies are summarized in the present paper.

Keywords: *Boerhaavia diffusa*, Ethnobotany, Phytochemistry, Pharmacology.

INTRODUCTION

The genus *Boerhaavia* L. (Family: Nyctaginaceae) consists of 40 tropical and sub-tropical species[1] found growing wild in different terrestrial habitats, ranging from managed grasslands, wastelands, agro-ecosystems to large forest gaps. The plants grow vigorously as weeds in tropical and subtropical regions such as India, Brazil, Africa, Australia, China, Egypt, Pakistan, Sudan, Sri Lanka, USA, Iran and in several countries of the Middle East. Out of these, six species of *Boerhaavia* are reported to occur in India – *B. diffusa*, *B. chinensis*, *B. erecta*, *B. repens*, *B. rependa*, and *B. rubicunda*[2-4]. *B. diffusa* L. was described as Punarnava (meaning one that rejuvinates the old body) in the *Atharvaveda*, as the top of the plant dries up during the summer season and regenerates again during the rainy season. The plant was named in honour of Hermann Boerhaave, a famous Dutch physician of the 18th century[2]. The whole plant of *B. diffusa* fresh or dried is the source of the drug punarnava which is official in Indian Pharmacopoeia as a diuretic.

B. diffusa L. is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, having spreading branches. The roots are stout and fusiform with a woody root stock. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at the nodes. Leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous above and usually white underneath; ovate-oblong, round or subcordate at the base with smooth, wavy, or undulate margins; up to 5.5 × 3.3 cm^2 in area. Flowers are minute, subcapitate, present 4–10 together in small bracteolate umbels, forming axillary and terminal panicles; hermaphrodite, pedicellate, and pink, or pinkish-red in color; Bracts are deciduous and involucrate; Calyx and corolla replaced by perianth which is tubular in shape, the tube being short and narrow at the base and funnel-shaped at the top and constricted above the ovary. There are five lobes, which are small and acute. Two or three stamens are present and are slightly exserted. The stigma is peltate. The fruit is an achene and is detachable, ovate, oblong, pubescent, five-ribbed and glandular, anthocarpous, and viscid on the ribs[5].

ETHNOBOTANY

Therapeutic uses: The root, leaves, aerial parts and the whole plant of *B. diffusa* L. syn. *B. repens* L. are used in different parts of India (Meghalaya, Rajastan, Orissa, Uttar Pradesh, Haryana, Gujarat etc.) as

well as worldwide for the treatment of a number of disorders e.g. liver complaint, kidney disorders, rheumatism etc (Table 1). The plant is bitter, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic, emetic, expectorant, anti-inflammatory, febrifuge and laxative besides being an active ingredient as a tonic. It is useful in all types of inflammation, strangury, leucorrhoea, ophthalmia, lumbago, myalgia, cardiac disorders, jaundice, anaemia, dyspepsia, constipation, cough, bronchitis and general debility[6]. The leaves are used for treatment of jaundice, liver complaints, hypotension, skin diseases (itches and eczema), night blindness and also used as an antidote to snake poisoning. Similarly roots are useful for gonorrhoea, dropsy, bronchial asthma, night blindness, rheumatism, several diseases of urine, liver, kidney and heart[7-12]. In Jajpur district of Orissa, India the plant decoction (1 teaspoon, twice daily) is given to parturient mothers in post delivery complaints such as body swelling[13]. Half table spoon of plant powder taken thrice a day with water relieves from menstrual trouble and its fruit paste along with paste of *Piper nigrum* fruits taken once daily for seven days cures cold[14]. Root decoction is used to treat fever[15]. It is reportedly used to treat internal inflammation of all kinds, dyspepsia, oedema, jaundice, cough, hemorrhoids, pulmonary cavitations, anaemia, enlargement of spleen, abdominal pain, abdominal tumours, cancers and acts as an antistress agent[16-17]. The root possesses anticonvulsant[18-19], expectorant, laxative, stomachic properties[20]. The root powder, when mixed with *mamira (Thalictrum foliolosum)* is used to treat eye diseases. It cures corneal ulcers and night blindness[21] and helps restore virility in men. People in different indigenous groups use it to hasten childbirth[22]. The juice of *Boerhaavia diffusa* leaves serves as a lotion in ophthalmia. It is also administered orally as a blood purifier and to relieve muscular pain[4]. *Boerhaavia diffusa* is used in renal ailments as diuretic[23-24] and to treat seminal weakness and blood pressure[25]. It is also used to treat stomach ache, anemia, cough, cold, used as a potent antidote for snake and rat bites and flowers and seeds are used as contraceptives[20]. Plant parts are applied as a stomachic, cardiotonic, hepatoprotective, laxative, diuretic, anthelmintic, febrifuse, expectorant and in higher doses as an emetic and purgative[26].

The entire plant including the roots is used as vegetable. In many parts of India such as Purulia (West Bengal), Assam etc. tribals cook *Boerhaavia* leaves as vegetables[27]. The roots and seeds are added to cereals, pancakes, and other foodstuffs[4]. Seeds are also served as bird feed. The plants are grazed by sheep, goats and cows and in West Bengal it is believed that the plant enhances lactation period and also the amount of milk in cattle[4].

Table 1: Ethnomedical uses of *Boerhaavia diffusa* by various countries

Name of the Country	Ethnomedical uses
Brazil	for albuminuria, beri-beri, bile insufficiency, cystitis, edema, gallbladder problems, gallstones, gonorrhea, guinea worms, hepatitis, hypertension, jaundice, kidney disorders, kidney stones, liver disorders, liver support, nephritis, renal disorders, sclerosis (liver), snakebite, spleen (enlarged), urinary disorders, urinary retention
Guatemala	for erysipelas, guinea worms
India	for abdominal pain, anemia, ascites, asthma, blood purification, cancer, cataracts, childbirth, cholera, constipation, cough, debility, digestive sluggishness, dropsy, dyspepsia, edema, eye problems, fever, gonorrhea, guinea worms, heart ailments, heart disease, hemorrhages (childbirth), hemorrhages (thoracic), hemorrhoids, inflammation (internal), internal parasites, jaundice, kidney disorders, kidney stones, lactation aid, liver disorders, liver support, menstrual disorders, renal insufficiency, rheumatism, snakebite, spleen (enlarged), urinary disorders, weakness, and as a diuretic and expectorant
Iran	for edema, gonorrhea, hives, intestinal gas, jaundice, joint pain, lumbago, nephritis, and as an appetite stimulant, diuretic and expectorant
Nigeria	for abscesses, asthma, boils, convulsions, epilepsy, fever, guinea worms, and as an expectorant and laxative
West Africa	for abortion, guinea worms, menstrual irregularities, and as an aphrodisiac
Philippines	Diuretic, fever, purgative and vermifuge
Ghana	Asthma and Boils
Elsewhere	for childbirth, guinea worms, jaundice, sterility, yaws

Phytochemistry

The quest to identify and isolate novel phytocompounds from *B. diffusa* has lead many researchers to discover various compounds such as flavonoids, alkaloids, glycosides, steroids, triterpenoids, lipids, lignans, carbohydrates, proteins, and glycoproteins from its leaves, stems, seeds and roots. Some of these are summarized in Table 2. Ghosal[28] was the pioneer to study and characterize the phytochemical properties of *B. diffua.* Later on a number of researchers isolated and characterized the active principles of *B. diffusa* extracts.

Chemical constituents of aerial parts

The physiochemical properties of leaves showed moisture content 84.5 %; protein 6.1 %; fat 0.9 %; carbohydrates 7.2 %; minerals 1.3g/100g; calcium 667.0; phosphorus 99.0; iron 18.4; vitamin C 27 mg/100g and energy 61 Kcal/100g. Ahuja[29] isolated as many as 15 amino acids, including 6 essential amino acids (total 9.43 %). These are: alanine 0.88; arginine 0.47; aspartic acid 0.69; glutamic acid 1.1; leucine 0.67; methionine 0.41; ornithine 0.67; phenylalanine 0.52; proline 0.35; serine 0.73; threonine 0.72; tryptophan 0.53; tyrosine 0.61; asparagines 0.33; glycine 0.75 and valanine 0.0%. Chopra *et al*.[30] reported that the plant contained large quantities of potassium nitrate and other potassium salts, besides the water soluble alkaloid punarnavine. Agarwal and Dutt[31] isolated boerhavic acid from the aerial portion. The main chemical constituents of *B. diffusa* are punarnavoside, boeravinone A-F, hypoxanthine 9-L arabinofuranoside, ursolic acid, liiriodendrin. Besides it also contain sterols[32], β-sitosterol[33-34], alkaloids[35], steroids and sugars[36]. A steroid androst-5-ene analogue and flavones, 6', 5'-dimethoxy-5, 7, 3-trihydroxyflavone was isolated from the aerial part[37]. Pereira *et al*.[38] identified four flavonoids derivatives (quercetin-3-O-robinobioside, eupalitin-3-O-galactosyl(1-2)-glucoside, kaempferol-3-O-robinobioside and eupalitin-3-O-galactoside), one aglycone, quercetin and two new volatile compounds camphor and safranal in leaves.

Table 2: Chemical constituents isolated from *Boerhaavia diffusa*

Class	Compounds	References
Alkaloid	Punarnavine	105, 106
		107
Rotenoids	Boeravinone A-F	42, 43
		108
	Hypoxanthine 9-L-arabinofuranoside	109
	Hentriacontane, β-sitosterol and ursolic acid	110
Glycoside	Punarnavoside	77, 111
	C-methylflavone 5,7-dihydroxy-3',4'-dimethoxy- 6,8-dimethylflavone	40
	β-ecdysone, triacontane	46
	β-sitosterol- β-D-glucoside	
Acids	tetracosanoic, hexacosanoic, stearic, palmitic, arachidic acids	112
	Boerhavin and boerhavic acid	113
Lignans	Liriodendrin	114
	syringaresinol mono- β- D-glucoside	44
	Glycoprotein	87
Lipids	5-methyleicos-4-ene	10
	Eicos-4-ene	
	4-methyloctadec-3-ene	
	4-methylnonadecylbenzene	
Phenolic compounds	3,4-dihydroxy-5-methoxycinnamoylrhamnoside	115
	Quercetin 3-*O*-rhamnosyl (1→6) galactoside (quercetin 3-*O*-robinobioside)	
	Quercetin 3-*O*-(2″- rhamnosyl)-robinobioside	
	Kaempferol 3-*O*-(2″-rhamnosyl)-robinobioside	
	3,5,4′- rihydroxy-6,7-dimethoxyflavone 3-*O*-galactosyl(1→2)glucoside [eupalitin 3-*O*-galactosyl(1→2)glucoside]	
	Caffeoyltartaric acid	
	Kaempferol 3-*O*-robinobioside	
	eupalitin 3-*O*-galactoside	
	Quercetin	
	Kaempferol	
	6, 9, 11-Trihydroxy-6a	116
	12a-dehydrorotenoid (coccineone B)	

Chemical constituents of Root

The root contains 14 amino acids, including 7 essential amino acids (total 11.54 %). These are: alanine 1.18; arginine 0.75; aspartic acid 0.95; glutamic acid 1.45; leucine 0.88; methionine 0.45; ornithine 0.96; phenylalanine 0.71; proline 0.5; serine 0.85; threonine 0.79; tryptophan 0.65; tyrosine 0.72; asparagines 0.0; glycine 0.0 and valine 0.75 %[29]. The root was reported to yield a new c-methyl flavone and dihydrofuranoxanthone designated as borhavone and borhavine respectively[39-40]. Four new compounds boerhavisterol, boerhadiffusene, diffusarotenoid and boerhavilanostenyl benzoate and a known rotenoid, boeravinone A were isolated from its roots[41]. Examination of the ether extracts of the roots of *B. diffusa* led to the isolation of boeravinone A, B[42], C[43], D, E, F[44], G and H[45]. A phytoecdysone named 'ecdysterone' which acts as an insect moulting hormone was also isolated from the root extracts of *B. diffusa*[46]. Roots presented fewer flavonoid derivatives quercetin-3-O-robinobioside and eupalitin-3-O-galactosyl (1-2)-glucoside, but exhibited one phenolic acid, caffeoyltartaric acid, which was absent in leaves[38].

Chemical constituents of whole plant

Characterization of the whole plant extract of *B. diffusa* led to isolation of four new flavonoids and phenol glycosides such as eupalitin 3-O-β-Dgalactopyranosyl-(1'''→2'')-O-β-D-galactopyranoside, 3,3',5-trihydroxy-7-methoxyflavone, 4',7-dihydroxy-3'-methylflavone and 3,4-dimethoxyphenyl-1-O-β-D apiofuranosyl-(1''→3')-O-β-D-glucopyranoside[47]. Two quinolizidine alkaloids identified as punarnavine I and punarnavine II was isolated from root, stem and leaves[48]. Several volatile compounds were identified in both root and leaves[38].

PHARMACOLOGICAL PROPERTIES

Anti-diabetic properties

The rapidly increasing diabetes mellitus and its associate complications is becoming a serious threat to human health. Its control and treatment mainly depend on the chemical or biochemical agents. The alternative medicines of herbal origin offer good clinical opportunities and show a bright future in the therapy of diabetes mellitus and its complications. *B. diffusa* has potent anti-diabetic activity. Its leaf extracts with various solvents have shown hypoglycemic activity in normal animals[49] and antihyperglycemic activity in alloxan,[49-50] streptozotocin[51] or dexamethasone[52] induced models of diabetes. Aqueous solution of *B. diffusa* leaf extracts at a daily oral dose of 200 mg/kg for 4weeks, showed significant ($p < 0.05$) change on blood glucose concentration and activity of hepatic gluconeogenic enzymes (increase in hexokinase activity and decrease in glucose-6-phosphate and fructose-1, 6- phosphate activity) in both normal and alloxan induced rats[49]. In another study chloroform extracts of *B. diffusa* leaves produced dose dependent reduction in blood glucose in streptozotocin induced non-insulin dependent *diabetes mellitus* in rats[51]. This was probably through rejuvenation of

pancreatic β-cells or through extra pancreatic action. Golap and Kar[52] reported the antiperoxidative, hypoglycemic and cortisol lowering activities of *B. diffusa* root extracts at a daily dose of 150 mg/kg in dexamethasone induced hypoglycemic rats and concluded its potential activity against corticosteroid induced diabetes mellitus.

Prashanth *et al*.[53] studied the α-amylase inhibitor activity of *B. diffusa* root extracts taking α-amylase inhibitors from *Phaseolus vulgaris* as active reference standard and found out that the root extract showed no *in vitro* α-amylase inhibition activity.

Immunomodulatory, immunosuppressive and anti-lymphoproliferative activity

Studies have shown immunosuppressive[54], immunomodulatory activities[55-56] and anti-lymphoproliferative activity[57] of various extracts, fractions and pure compounds of *B. diffusa.* Pandey *et al*.[54] observed that the chloroform (50 µg/ml) and ethanol extracts 50 µg/ml) and the pure compound eupalitin- 3 O-β- D- galactopyranoside (500 µg/ml) inhibited PHA stimulated proliferation of PBMCs, two-way MLR, NK cell cytotoxicity as well as LPS induced nitric oxide production by RAW 264.7 and the hexane extract (100 µg/ml) and eupalitin (100 µg/ml) showed suppression of mitogen (PHA) stimulated proliferation of PBMCs. Mungantiwar *et al*.[55] observed delayed hypersensitivity in mice immunized intraperitoneally with sheep RBC after oral administration with 25-100 mg/kg of the alkaloid fraction for 10 days around immunization. They also observed a dose related increase in humoral antibody titres in those mice. The alkaloid fraction failed to show any blastogenic effect (lymphocyte proliferation) responsiveness of murine splenocytes to the T-cell mitogen Con A and the B-cell mitogen lipopolysaccharide (LPS). *In vitro* studies, Mehrotra *et al*.[56] observed that the ethanolic plant extract (100 and 500 µg/ml) inhibited human NK cell cytotoxicity, lipopolysaccaride induced nitric oxide production in mouse macrophase cells RAW 264.7. At a concentration as low as 10 µg/ml it inhibited phytohaemoagglutinin stimulated IL-2 as well as lipopolysaccharide stimulated TNF- α production in human PBMCs culture and inhibited cytokine production. Mehrotra *et al*.[57] also observed a stimulatory effect on antigen stimulated PBMC proliferation and on human mixed lymphocyte culture and observed an inhibitory effect on growth of various cell lines of mouse and human origin. Manu and Kuttan[58] observed the effect of the purified alkaloid punarnavine (40 mg/kg) on immune system and found out enhanced WBC count, increase in bone marrow cellularity and number of alpha-esterase positive cells, enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in the presence and absence of specific mitogens *in vitro* and *in vivo* and reduction in LPS induced elevated levels of proinflammatory cytokines.

Hepatoprotective activity

B. diffusa has long been used as a popular hepatoprotective medicine[59-61]. Various extracts of the aerial part and roots of this plant showed hepatoprotection against CCl_4[37, 62] country made liquor[63], thioacetamide[64] and acetaminophen-induced[65] hepatotoxicity in rats. The hepatoprotective action was evident from the reduction in the increased levels of serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) in the treated rats. Chakraborti and Handa[37] reported hepatoprotective activity of a steroid androst 5-ene analogue (200 µg/ml) and a flavone 6, 5'-dimethoxy- 5, 7, 3'-trihydroxyflavone (50 µg/ml) isolated from the aerial parts against CCl_4 intoxication in *in vitro* models. Rawat *et al*.[64] observed protective effect of 2ml/kg of aqueous extract and 150 mg/kg of powdered form of the thin roots in thioacetamide intoxicated albino rats.

Analgesic activity

Boerhaavia diffusa is used in 'Martinican folk medicine' for its analgesic and anti-inflammatory properties. Considering this Hiruma-Lima *et al*.[66] evaluated the plants potential for antinociceptive effect. They used a lyophilized decoction (DE) and fresh leaf juice (JE) to determine their antinociceptive effect using acetic acid induced abdominal writhing (chemical) model and hot plate (thermal) analgesic test, taking dipyrone (10 ml/kg, *p.o.*) and morphine (10 mg/kg, s.c.) as positive controls respectively. Significantly inhibition was observed in acetic acid induced abdominal writhing in mice, when compared with negative control animals. In the thermal model of hot plate test showed that oral administration of JE raised the pain threshold at observation times 60 and 90 min. whereas DE had a short-lasting effect, significantly increasing the latency only at 30 min. The researchers were also tried to determine the possible mechanism underlying this analgesic activity. For this they determined the effect of JE and DE on the sleeping time induced by pentobarbital sodium, a neurosedative in mice. Administration of JE and DE 30 min before an intraperitoneal dose of pentobarbital sodium did not increase the latency or duration of sleeping time when compared with saline treated group. These results raised the possibility that the reduction in acetic acid induced writhing and protection on the hot plate by DE and JE did not result from a sedative property but from their analgesic activity. They also evaluated the anti-inflammatory activity of JE and DE against carrageenan induced pedal oedema in mouse. They found that neither JE nor DE at the dose of 1000 mg/kg significantly decrease paw swelling compared to reference drug indomethacin indicating the lack of a peripheral analgesic property. They also indicated that morphine receptor antagonist like naloxone can modify the analgesic effect of JE but not DE. This indicated that this effect results due to action on opioid receptors or the release of endogenous opioid substances. So they concluded that the antinociceptive property of the extracts of *B. diffusa* is due to the existence of a central analgesic property.

Borrelli *et al*.[67] found that methanol extract obtained from roots of *B. diffusa* was able to inhibit the contractions induced by acetylcholine (ACh) in the isolated guinea pig ileum. A detailed phytochemical analysis of this methanol extract led to the isolation of a new compound 9-*O*-methyl-10-hydroxycoccineone B and six known rotenoid derivatives such as boeravinone D, boeravinone E, 6-*O*-demethylboeravinone H, boeravinone H, boeravinone G, boeravinone C, 10-demethylboeravinone C, coccineone E, 2'-*O*-methylabronisoflavone, boeravinone F and coccineone B.

Anti-cancer properties

Various preliminary studies (*in vitro* as well as *in vivo*) with crude plant extract and its various purified fraction have shown anticancer activity against pulmonary metastases[68]. Bharali *et al*.[69] demonstrated its chemopreventive action against 7, 12- dimethyl benz(a)anthracene (DMBA) induced skin papillomagenesis and Manu *et al*.[70] demonstrated the protective activity of the hydro-alcoholic extract of the plant against gamma radiation induced damage. Sreeja and Sreeja[71] observed the antiproliferative and antiestrogenic properties of methanol extract of *Boerhaavia diffusa* in MCF-7 breast cancer cell lines suggesting its therapeutic role in estrogen induced breast cancers.

Inhibitory effect on experimental metastases

Leyon *et al*.[68] studied the inhibitory effect of hydro-alcoholic extract of *B. diffusa* on experimental pulmonary metastases formation by B16F10 melanoma cells in male C57BL/6 mice. It was shown that prophylactic (0.5 mg/dose, *i.p.*) and simultaneous administration of the extract inhibited metastases formation by about 95% and 87% respectively as compared to untreated control animals. In the control animals the biochemical parameters such as lung collagen hydroxylproline, hexosamine and uronic acid levels were drastically elevated compared to the normal levels, whereas in the treated groups the levels were found to be reduced along with reduction in levels of serum sialic acid and serum γ-glutamyltranspeptidase activity that are markers of neoplastic proliferation. The survival rate of the treated

animals increased (more than double) with reduced fibrosis and smooth alveolar function than that of untreated control animals. When a non-toxic concentration of the extract was treated directly to the B16F10 cells *in vitro* it inhibited the cell proliferation as estimated by the ^{3}H-thymidine uptake assay. From the zymogram analysis using culture supernatant from the extract treated cells it became evident that the components of the extracts inhibited the expression or activity of gelatinases A and B (MMP-2 and MMP-9). As MMPs are closely associated with cell invasion and angiogenesis, inhibition of these functions along with anti-proliferative activity may be attributed to the antimetastatic property of *B. diffusa*. Administration of Punarnavine (40 mg/kg 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[58].

Manu and Kuttan[72] observed enhanced immune response against metastatic progression of B16F-10 melanoma cells in mice on administration of the alkaloid punarnavine. This was evident from the enhanced Natural Killer (NK) cell activity, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent complement mediated cytotoxicity (ACC), enhanced production of the cytokine IL-2 and lowered levels of GM-CSF and pro-inflammatory cytokines such as IL-1beta, IL-6 and TNF-a in treated group compared to the metastatic tumor bearing control. Such enhanced levels of cell mediated immune response were also observed with the crude extract of *B. diffusa* against metastatic progression of B16F-10 melanoma cells in mice[73].

Chemopreventive action against skin carcinogenesis

Bharali *et al.*[69] demonstrated the chemopreventive property of *B. diffusa* extract on the two-stage mechanism of 7, 12- dimethyl benz(a)anthracene (DMBA) induced skin papillomagenesis in male swiss albino mice (6-7 wk old). A sub minimal dose of DMBA (50 µg/µl of acetone) was applied to initiate tumorigenesis followed 2 weeks later by repeated application of croton oil (1 % in acetone three times a week) to promote development of tumor to a visible stage. Extract of *B. diffusa* was applied topically on the shaven backs at 3 different stage of tumorigenesis ie peri-initial phase (7 days before and 7 days after the application of DMBA; Group II), promotional stage (from the day of start of croton oil treatment and continued till the end of experiment; Group III) and peri as well as post-initial phase (7 days prior to DMBA application and continued till the end of experiment; Group IV). A significant reduction in the values of tumor incidence (Group II- 65%; Group III- 30%; Group IV- 25%), average number of tumors per tumor bearing mouse (Group II- 2.8; Group III- 0.75; Group IV- 0.35) and papillomas per papilloma bearing mouse (Group II- 3.1; Group III- 2.5; Group IV- 1.2) were observed. According to the authors the inhibitory activity of the plant extract may be 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.

Protective effect against gamma radiation induced damage

The protective effect of the hydro-alcoholic extract of the whole plant of *B. diffusa* was studied against gamma radiation induced damage in mice[70]. Ionizing radiations though helpful in treatment of cancer, has lethal side effects like immunosuppression. *B. diffusa* with its established immunomodulatory activity was proved to be a good radioprotector. Administration of 20 mg/kg of *B. diffusa* extract intraperitoneally effectively sustained the level of total WBC count in the blood of treated animals. A similar correction was also observed in the case of bone marrow cellularity and α-esterase-positive cells. The lower levels of alkaline phosphatase (ALP) and glutamate pyruvate transaminase (GPT) indicated the hepatoprotective effect of *B. diffusa* in gamma irradiated mice. In *B. diffusa* treated mice the enhanced GSH in intestinal mucosa protects it from radiation damage. Treatment with *B. diffusa* extract reduced the DNA fragment caused by gamma radiation with near recovery within 24 hours.

Anti-proliferative and antiestrogenic properties in breast cancer cell lines

Sreeja and Sreeja[71] observed the antiproliferative and antiestrogenic properties of methanol extract of *Boerhaavia diffusa* in MCF-7 breast cancer cell lines using MTT assay, Hydroxylapatite assay (HAP). Treatment with varying concentrations of the extract (20–320 µg/mL) resulted in growth inhibition in MCF-7 cell lines. BME competed with [^{3}H]-estradiol for binding to estrogen receptors with IC_{50} value of 320 ± 25 µg/mL. RT-PCR analysis revealed that the extract reduced the mRNA expression of *pS2* indicating its the antiestrogenic action. The extract treatment for 48 h resulted in a remarkable alternation in the cell cycle phases. This increased the number of MCF-7 cells in the G0-G1 phase from 69.1% to 75.8%, with a reciprocal decrease of cells in all other phases indicating cell cycle arrest at G0-G1 phase.

Cytotoxic activity

Srivastava *et al.*[74] evaluated and compared the cytotoxic activity of ethanolic extract of *B. diffusa* roots and leaves with standard anticancer drug, methotrexate, by MTT assay and with trypan blue dye exclusion method. *In vitro* screening of the extract of *B. diffusa* indicated the crude fraction appeared to be cytotoxic against tumor cells. A dose dependent cytotoxic effect of root and leaf extract was compared with methotrexate. Methotrexate showed almost 40% cell death at a concentration of 200nM, whereas crude ethanolic extract of root showed almost 30% cell death at concentration of 200µg/ml; alkaloid fraction and leaf extract each showed 40% cell death at 300µg/ml. This showed that the plant has promising cytotoxic activity.

Non-teratogenic in action

Singh *et al.*[75] (1991) in an attempt to evaluate any possibility of teratogenic effects in *B. diffusa* administered its ethanolic extract daily in an oral dose of 250 mg/kg of body weight to pregnant albino female rats during the entire period of gestation. They found that it was devoid of any teratogenic effect as litter size and survival rate of foetuses were the same as for the normal control group and no foetal anomaly could be detected.

Anti-fibrinolytic activity

Intrauterine devices (IUDs) used for contraception resulted in *in vitro* fibrinolytic activity in the uterus. Local fibrinolysis increases levels of plasminogen activators resulting in uterine hemorrhage and excessive menstrual blood and iron loss. Studies using 50% freeze dried ethanolic extract (conc. 150 mg/ml) of *B. diffusa* showed to inhibit *in vitro* fibrinolytic activity on fibrin plates in the copper-IUD fitted rabbits. Similarly, increased fibrinolytic activity of human fallopian tube when tested histochemically on fibrin film was found to be inhibited by aqueous extract of *B. diffusa* (80 mg/0.06 ml)[76]. The root extracts produced noticeable reduction in the amount and duration of menstrual flow (124%) and menstrual iron loss[77] and reduced activity of uterine tissue plasminogen activator (tPA)[78] in IUD fitted monkeys. Root extracts of *B. diffusa* was found most effective in reducing stromal edema, inflammation, tortuosity of glands, and in increasing the degree of deposition of fibrin and platelets in the vessel lumen as revealed by endometrial histology of IUD-fitted menstruating monkeys[79].

Antioxidant activity

Satheesh and Pari[80] demonstrated that administration of *B. diffusa* leaf extract (200 mg/kg, *p.o.*) for 4 weeks resulted in a significant reduction in thiobarbituric acid reactive substances and

hydroperoxides, with a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase in liver and kidney of alloxan induced diabetic rats. The results suggested that BLEt can remarkably improve antioxidant status in alloxan induced diabetic rats. Pereira *et al*.[38] analysed the volatile composition of *B. diffusa* (leaves and roots) using HS–SPME–GC–MS and identified several compounds, including terpenes, phenylpropanoids, indol compounds, orisoprenoids, among others. 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. Additionally, several flavonoids and one phenolic acid were also confirmed. With the fact that volatile compounds are described to have several biological functions, such as antioxidant, antiseptic or anti-atherosclerotic[81], they evaluated the anti-oxidant activity of the aqueous extract against DPPH radical, one reactive oxygen species (O_2^-) and one reactive nitrogen species (NO) and anti-acetylcholinesterase activity. Leaves revealed stronger DPPH radical and nitric oxide radical scavenging activity than roots, presenting an IC_{25} of 41 lg/mL and 1234 lg/mL, respectively and IC25 of 155 and 217 lg/mL, respectively. However roots revealed stronger superoxide radical scavenging activity than leaves presenting an IC_{50} of 45 and 297 lg/mL respectively. No acetylcholinesterase inhibition was found in either plant parts.

Anti-viral activity against plant viruses

Extracts from the roots of *B. diffusa* L., stems of *Cuscuta reflexa* Roxb. and leaves of *Euphorbia hirta* L. have shown a potential protective effect on the infection of potato virus X, in hypersensitive and systemic hosts. The inhibition by these extracts was systemic and sensitive to actinomycin D[82]. The destructive yellow mosaic disease of mungbean (*Vigna radiata*), caused by mungbean yellow mosaic virus, was controlled by the application of the aqueous root extract of *B. diffusa*. Six sprays of *B. diffusa* root extract were found most effective, as it considerably delayed symptom appearance, suppressed symptom severity and decreased disease incidence by 80-90%. This treatment also increased root nodulation, plant height, primary and secondary branches, pod formation and grain yield[83].

The purified glycoprotein (70 - 80% protein and 8–13% carbohydrates; Mol. Wt 16 – 20 kDa) from *B. diffusa* exhibited strong antimicrobial activity against RNA (ribonucleic acid) bacteriophages[84]. The roots of *B. diffusa* are a rich source of this protein, which is used for inducing systemic resistance in many susceptible crops against commonly occurring viruses[85-90]. This protein or antiviral agent was active against tobacco mosaic virus in *Nicotiana glutinosa*, *Datura metel*, *Chenopodium amaranticolor*, and *Nicotiana tabacum* (Ky58 White Burley and NP31); sunhemp 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[85,88].

Anti-bacterial activity

Studies showed that the aqueous and methanolic extracts of *B. diffusa* has significant anti bacterial activity against a number of human pathogenic bacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella* sp., *Staphylococcus aureus* and *Yersinia enterocolitica* at 50µl concentration[91]. Howevr, no MIC value of the extract against each bacterium was determined.

Anti-fungal activity

Antifungal activity of various extracts (pt. ether, chloroform, ethyl acetate, ethyl alcohol and aqueous) of aerial and root parts of *B. diffusa* was screened against dermatophytic fungi *Microsporum gypseum*, *M. fulvum* and *M. canis*[92-93]. Extracts of aerial parts failed to show any noticeable antifungal activity. Ethyl acetate extract of roots of the plant was found to be most effective against target fungal species. The maximum inhibition of mycelial growth was observed for *M. gypseum* (78.83%) followed by *M. fulvum* (62.33%) and *M. canis* (42.30%) of ethyl acetate in the test concentration of 1000 µg/ml 24 hours of incubation. Qureshi *et al*.[94] evaluated the inhibitory nature of extracts of 18 plant species of Chhindwara including *B. diffusa* against 3-Keratinophilic fungi *Microsporum gypseum*, *Chrysosporium tropicum* and *Trichophyton terrestre*. *B. diffusa* showed inhibition against all the three fungi tested. Sankar and Sreeramulu[95] have shown the inhibitory effect of hot water extract of leaves of *B. diffusa* against the powdery mildew fungus, *Uncinula tectonae* infecting *Tectona grandis*.

Adoptogenic activity

Adaptogens seem to be useful during both adrenal hyperstress as well as adrenal hypofatigue. By definition, an adaptogen implies the capability for bi directional or normalizing effects. Mungantiwar *et al*.[96] studied the adaptogenic potential of aqueous extract of *B. diffusa* root powder by studying its effect on *Escherichia coli*-induced abdominal sepsis, macrophage phagocytic activity in mice and on cold and forced swimming stress in rats. Administration of a daily dose of 200mg/kg of plant extract orally for 15 days prior to *E. coli* challenge produced significant leucocytosis with reduction in macrophage phagocytic activity in mice. The plant extract reduced the levels of glucose, cholesterol, serum glutamate pyruvate transaminase and triglycerides, whose levels were otherwise elevated due to stress. The alkaloid fraction isolated from the plant showed 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 rats.

Anti-convulsant activity

B. diffusa was found to be widely used in epilepsy in Nigerian folk medicine[97]. The compound 'liriodendrin' isolated from the methanolic root extract of this plant was reported to show a calcium channel antagonistic activity[43-44]. Basing on these facts Kaur and Goel[98] verified the anti-convulsant activity of various root extracts of *B. diffusa* in male Swiss albino mice. They took the methanolic extract (1000, 15000 and 2000 mg/kg), the liriodendrin rich fraction (10, 20 and 40 mg/kg) of this extract, chloroform fraction (20 mg/kg) and phenolic compound fraction (1 mg/kg) for the test. For preparation of dose a weighed quantity of the extract and its fractions were dissolved in dimethylsulfoxide (DMSO) and the resultant solution was dispersed in 0.5 % CMC (DMSO 1: CMC 9) freshly before use and was injected intraperitoneally. Pentylenetetrazol (PTZ; 75 mg/kg) injected intraperitoneally to induce convulsion because calcium ion was a common mediator in PTZ induced convulsions. To test the calcium channel antagonistic activity the animals were administered with the liriodendrin rich fraction 30 min prior to the intracerebroventricular injection of BAY k-8644. There was a significant ($P<0.05$) delay in the onset of convulsions and reduction in mortality at all the three doses of methanolic extract (1000, 1500 and 2000mg/kg) in PTZ model, as compared with control group. The chloroform fraction and phenolic compound fraction showed no protection against PTZ-induced convulsions and complete mortality was observed in all of these groups. Among the different fractions liriodendrin rich fraction showed a significant ($P<0.05$) delay in the onset of convulsions and no mortality in PTZ model in a dose-dependent manner showing maximum protection at 40mg/kg dose. Pretreatment with liriodendrin-rich fraction showed protection against BAY k-8644-induced seizures. Liriodendrin-rich fraction-treated groups showed a significant ($P<0.05$) delay in the onset and reduction in the severity of seizures induced by BAY k-8644 in a dose-dependent manner as compared with control group. The authors concluded that the observed anti-convulsant activity was due to its calcium channel antagonistic action as this activity was retained only

in the liodendrin-rich fraction, which had additionally been confirmed by significant anti-convulsant activity of liriodendrin-rich fraction in BAY k-8644-induced seizures.

Effect on semen and testicular morphology

Adenubi *et al*.[99] studied the effect of oral administration of the aqueous extract of the leaves of *B. diffusa* (50, 100 and 150mg/kg) for 60 days on semen and testicular morphology in male Wistar rats. The serum testosterone level, weights and histopathology of the testes, epididymes and seminal vesicles were determined and the spermatozoa from the cauda epididymes of the groups were studied. No significant difference was found in the serum testosterone level between treated and control rats. A significant decrease ($P<0.05$) was observed in the weights of the testes, epididymes and seminal vesicles in treated rats compared with that of the control. Sperm motility and sperm count decreased significantly ($P<0.05$) also in the treated rats compared with that of the control. In addition, the sperm live-dead ratio decreased significantly ($P<0.05$) in rats treated with 100 and 150mg/kg of the extract compared with the control. Histopathology of the testes of treated rats revealed marked degeneration of germinal epithelia with spermiostasis. This result suggests that aqueous extract of *B. diffusa* produced adverse effects in semen and testicular morphology of the rats.

Effect on crystal dissolution

In a study conducted by Raut *et al*.[100] on crystal dissolution activity of alcoholic and hydroalcoholic extracts of *B. diffusa* (500 µl) roots on crystals of monosodium urate monohydrate, calcium pyrophosphate monohydrate and basic calcium phosphate, the extracts failed to dissolute the crystals of monosodium urate monohydrate.

Clinical trials

Supporting the pre clinical studies several clinical studies were carried out in *B. diffusa* extracts. These are as follows

In treatment of pulmonary tuberculosis

Tuberculosis is a common and often deadly infectious disease. Its treatment is difficult and requires long courses of multiple antibiotics. In multi-drug-resistant tuberculosis, antibiotic resistance is becoming a growing problem. So it's necessary to found alternative methods to curb this problem. Surya *et al*.[101] used Punarnava (*B. diffusa*) as an adjuvant in the treatment of 25 patients of pulmonary tuberculosis in addition to chemotherapy. A group of 25 patients of pulmonary tuberculosis were maintained as control and received only chemotherapy. The patients were followed for 2 months. Results revealed that the group of patients who received Punarnava along with chemotherapy showed significantly faster and earlier clinical recovery, radiological clearing, sputum conversion and more weight gain and increase in T lymphocyte count as compared to those who received chemotherapy alone.

Anthelminthic activity

Singh and Udupa[102] reported that dried root powders of *Boerhaavia diffusa* showed curative efficiency against helminth infections. They found children or adults suffering from helminth infection became worm-free within five days when administered orally with the powder.

Toxicology

Several toxicological studies were conducted on *B. diffusa*. Hiruma-Lima *et al*.[66] studied the acute oral toxicity of a crude extract obtained from a lyophilized decoction (DE) and from the juice (JE) of fresh leaves. No sign of toxicity was seen upto a dose of 5000 mg/kg of body weight in mice and also no body or organ weight gain was also observed. Dhar *et al*.[103] indicated the maximum tolerated dose of root extract injected into mice to be approximately 1g/kg of body weight. A daily dose of 250 mg/kg of ethanolic extract of *B. diffusa* administered to pregnant albino female rats showed no teratogenic activity[76]. Chandan *et al*.[104] observed a strong choleretic action, resulted in an increase in bile flow and no signs of toxicity up to an oral dose of 2g/kg body weight in mice.

CONCLUSION

B. diffusa is a well-known medicinal plant that is frequently prescribed in various indigenous systems of medicine such as *Ayurveda* and *Unani*. The plant has a number of traditional uses for ameliorating multiple diseases, which were further supported by several pharmacological and clinical studies detailing the specific bioactivity of extracts of the plant. Most of the mentioned studies have been conducted using crude preparation of *B. diffusa* roots or leaves and the chemical profile was not mentioned. However, few studies have demonstrated biological activity of *B. diffusa* compounds such as the punarnavine, liriodendrin, glycoprotein, eupalitin- 3 O-β- D-galactopyranoside and eupalitin. The leaf and root extracts of *B. diffusa*, as reviewed here, have been found to have antimicrobial activity against several bacteria, fungi, viruses and parasites. The activity guided purification resulted in the isolation of a glycoprotein (70 - 80% protein and 8–13% carbohydrates; Mol. Wt 16 – 20 kDa) exhibiting strong antimicrobial activity against RNA (ribonucleic acid) bacteriophages. Research summarised here also indicates that the plant extracts exhibit significant immunomodulatory, immunosuppressive and anti-lymphoproliferative activities. Pharmacological studies showed that it possesses diuretic and anti-inflammatory activities, which makes it more suitable in the treatment of inflammatory renal diseases. It also exhibits antioxidant and anti-inflammatory effects as oxidative injury underlies many of the diseases. In other animal studies plant extracts showed anti-diabetic, anti-fibrinolytic, analgesic, antioxidant and adoptogenic activities. However, the diverse pharmacological activities of the plant extract and isolated phytochemicals have only been assayed in *in vitro* tests using laboratory animals, and very few clinical studies were available. So, the results obtained may not necessarily be portable to the situation in humans. There is minimal knowledge available of its uses traditionally in tumors or cancers, experimental results revealed that the plant *B. diffusa* has significant cytotoxic activity against tumor cells, which should be further explored for identification of new antineoplastic agents. It inhibits experimental metastasis, chemopreventive against skin cancer and also gives protection in radiation induced damage. Extracts of *B. diffusa* showed no adverse side effects or toxicity, so can be used for a long period of time. However studies have established several delayed toxicities of anti-tumor therapy on long term survivors. In the light of the potential role of *B. diffusa* as an anti-metastatic agent and its use in Ayurvedic medicine, a further toxicity test on the plant especially during prolonged administration is essential.

While there are gaps in the studies conducted so far, which need to be bridged in order to exploit the full medicinal potential of *B. diffusa*, it is still very clear that this is a plant with tremendous widespread use now and also with extraordinary potential for the future. This review is the collection of the studies conducted on various aspects on *B. diffusa* by different authors and the traditional healers to provide useful information for future scope of research and for conservation of this valuable species.

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