The Chemical Constituents and Pharmacological Effects of Bryophyllum calycinum. A review

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Abstract:

Bryophyllum calycinum belongs to the family crassulaceae was widely used in traditional medicine especially in the tropical areas. The plant contained alkaloids, phenols, flavonoids, tannins, anthocyanins, glycosides, bufadienolides, saponins, coumarins, sitosterols, quinines, carotenoids, tocopherol and lectins . The previous pharmacological studies showed that it exerted many pharmacological effects including anticancer , antioxidant immunomodulating , antibacterial , anthelmentic , antiprotozoal , neurologica (sedative and anticonvulsant) , anti-inflammatory , analgesic , diuresis , antiurolithitic , nephroprotective, hepatoprotective , anti-peptic ulcer , hypotensive , antidiabetic , wound healing and other pharmacological effects. The present review was designed to highlight the chemical constituents and pharmacological effects of *Bryophyllum calycinum*.

Keywords: Bauhinia variegata , Constituents **,** *Pharmacology* **,** Physicochemical

Introduction:

Bryophyllum calycinum (Synonyms: *Kalanchoe pinnatum*, *Bryophyllum pinnatum*) [(1)] , belongs to the family crassulaceae was commonly known as sprouting leaf . It was found in the tropical parts, southern Africa, and American [(2)]. The leaves and leaf juice of the plant were used traditionally as antiviral, antipyretic, antimicrobial , anti-inflammatory , antitumor , hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, antidiabetic, astringent, antiseptic, antilithic and cough suppressant[(3-11)]. Phytochemical studies showed that the plant contained alkaloids, phenols, flavonoids, tannins, anthocyanins, glycosides, bufadienolides, saponins, coumarins, sitosterols, quinines, carotenoids, tocopherol and lectins [(12-19)]. The pharmacological studies showed that it exerted many pharmacological effects including anticancer , antioxidant immunomodulating , antibacterial , anthelmentic , antiprotozoal , ,neurologica (sedative and anticonvulsant) , anti-inflammatory , analgesic , diuresis , antiurolithitic , nephroprotective, hepatoprotective , anti-peptic ulcer , hypotensive , antidiabetic , wound healing and other pharmacological effects. The present review aimed to provide detail information regarding the chemical constituents and pharmacological effects of *Bryophyllum calycinum*.

Physicochemical properties:

Total ash 5.1% , acid insoluble ash 1.69% , water soluble ash 4.19% , water soluble extractive value 19.80%, and alcohol soluble extractive value 5.60%[(12)].

Chemical constituents:

Phytochemical studies showed that the plant contained alkaloids, phenols, flavonoids, tannins, anthocyanins, glycosides, bufadienolides, saponins, coumarins, sitosterols, quinines, carotenoids, tocopherol and lectins [(13-19)]. Protein nitrogen and soluble nitrogen in the excised leaves of *Bryophyllum calycinum* were 0.97- 1.38 and 0.37- 0.81 g /kg respectively [(20)].

Leaves of *Bryophyllum calycinum* contained many acids including malic acid, isocitric acid, oxalic acid and succinic acid [(21)]. Threo -D_s-isocitric acid as the mono-potassium salt of the lactone was also isolated from dried *Bryophyllum calycinum* leaf tissue. The overall yield was 49 to 62% of the isocitric acid in the organic acid extract [(22)]. However , syringic acid, caffeic acid, 4-hydroxy-3-methoxy-cinnamic acid, 4- hydroxybenzoic acid, p- hydroxycinnamic acid, paracoumaric acid, ferulic acid, protocatechuic acid, phosphoenolpyruvate, protocatechuic acid were also isolated from aerial parts of plants[(17,23-24)]. The leaves contained flavones, falvans, flavanones, isoflavonoids, chalcones, aurones , anthocyanidines , 5 Methyl 4, 5, 7 trihydroxyl flavone 1 , 4, 3, 5, 7 tetrahydroxy 5-methyl 5I-propenamine anthocyanidines2 , 24-epiclerosterol (R)-stigmasta-5, 25-dien-3β-ol], 24(R)- 5α-stigmasta-7, 25-dien-3β-ol, 5α-stigmast-24-en-3β-ol and 25-methyl-5α-ergost-24 (28)-en-3β-ol, 1-octane3-O-α-L-arabinopyranosyl-(1-6)-glucopyranoside , isorhamnetin-3-O-a-L-1C4-rhamnopyranoside, 40-methoxy-myricetin-3-O-a-L 1C4-rhamnopyranoside and protocatechuic-40-O-b-D-4C1-gluco-pyranoside , bersaldegenin- 1, 3, 5-orthoacetate , bufadienolide- bryophyllin B , bryophyllin C , Stigmast-4, 20 (21), 23-trien-3-one , stigmata-5-en-3β-ol , α – amyrin- β-D-glucopyranoside and nundecanyl n-octadec-9-en-1-oate and n-dodecanyl noctadec- 9-en-1-oate[(25-31)]. The plant also contained calcium(96.45

µg/g of crude drug) , potassium (76.40 µg/g of crude drug), phosphorus, sodium, magnesium ,iron , zinc , ascorbic acid (26.42 to 44.03 mg/100 g), riboflavin (0.20 to 0.42 mg/100 g), thiamine (0.11 to 0.18 mg/100 g), niacin (0.02 to 0.09 mg/100 g), casein hydrlylsate and nicotinamide[12,30,32-33] .

Pharmacological effects:

Anticancer effects:

The antitumor effect of *Bryophyllum calycinum* Salisb. was evaluated against Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The effect of methanol and aqueous extracts of *Bryophyllum calycinum* on tumor growth was evaluated by , the percentage inhibition of ascetic cells and percentage inhibition of tumor weight. Methanol and aqueous extracts were administered at doses of 100,200 and 400 mg/kg body weight intraperitonially once a day for 7 days, after 24h of tumor inoculation. Decreases in tumor cell count and tumor weight were observed in extract treated animals when compared to EAC treated animals. The results were dose dependent in case of methanol extract [2]. Five bufadienolides isolated from the leaves of the plant were examined for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells induced by the tumor promoter,12- Otetradecanoylphorbol-13-acetate. All bufadienolides showed inhibitory activity, and bryophyllin A exhibited the most marked inhibition among the tested compounds. Bryophyllin C and bersaldegenin-3-acetate were less active [34].

Immunomodulatory effects:

Mice treated daily with oral *B.pinnatum* during hypersensitization with ovalbumin were protected against death. Oral protection was accompanied by a reduced production of OVA-specific IgE antibodies, reduced eosinophilia, and impaired production of the IL-5, IL-10 and TNF- α cytokines. Oral treatment with the quercitrin flavonoid isolated from plant extract prevented fatal anaphylaxis in 75% of the animals. These findings indicated that oral treatment with *Bryophyllum pinnatum* effectively downmodulates pro-anaphylactic reactions inducing immune responses [35]. The aqueous extract of leaves causes significant inhibition of cell-mediated and humoral immune responses in mice .The spleen cells of animals pretreated with plant extract showed a decreased ability to proliferate in response to both mitogen and antigen in vitro as well as, the specific antibody responses to ovalbumin were also significantly reduced by treatment[36].

Antimicrobial effects:

The antimicrobial effects of petroleum ether, chloroform, methanol and aqueous extracts was evaluated in *vitro* against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. Methanolic extract of roots *was* found to be most effective antibacterial compared to others, while none of extract showed the activity against *C. albicans* [37].

Agar cup plate test was used to determine the sensitivity of the tested *Bryophyllum calycinum* Salisb leaf extracts and the micro-dilution method was used to determine the minimum inhibitory concentration. The aqueous extract was active against all tested microbial strains (Gram-positive *:Staphylococcus aureus* ATCC 25925, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermis* ATCC 12228 and *Micrococcus luteus* ATCC 10240 ; and Gram-negative : *Enterobacter aerogens* ATCC 13048, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 51812 and *Shigella dysenteriae* ATCC 25931). The aqueous extract showed antimicrobial activity against all tested microorganism with minimum inhibitory concentration ranging between 0.26 to 2.08 mg/ml, while , the MICs of alcoholic extract ranged between 1.04 to 8.32 mg/ml [1]. Flavonoids , (5 methyl 4,5,7 trihydroxyl flavone and 4,3,5,7 tetrahydroxy 5-methyl 5-propenamine anthocyanidines) possessed significant antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia, E.coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*[30,38].

Anthelmentic and antiprotozoal effects:

The crude extracts contained tannins which produced anthelmentic activity. The chloroform, methanolic and aqueous extract of the plant root cause paralysis and deaths of worms and showed significant anthelmentic activity [12, 39]**.** The antileishmanial effect of the plant extracts and its flavonoids components was evaluated in *vivo* in murine model of cutaneous leishmaniasis. Quercetin 3- O-α-L-arabinopyranosyl, α-L-rhamnopyranoside, quercetin 3-O-α-L- rhamno pyranoside and free quercetin were able to control the lesion growth caused by *Leishmania amazonensis* and significantly reduce the parasite load. These flavonoids were as effective as the crude aqueous extract which indicated that the antileishmanial effect could be attributed to flavonoids [40-41].

Antioxidant effects:

The DPPH and nitric oxide free radical scavenging method were used to detect oxidative activity of *Bryophyllum calycinum* Salisb leaf extracts. The results of DPPH method showed 50% inhibition rate at the 144.23µg/ml and 117.42µg/ml with aqueous and alcoholic extract, respectively. Nitric oxide scavenging inhibition showed 50% inhibition rate at the 525.92µg/ml and 460.48µg/ml with aqueous and alcoholic extract, respectively[1].

Neuropharmacological effects:

Sedative effects:

The methanolic extract of *Bryophyllum calycinum* Salisb showed neuropharmacological effects in experimental animals (rats and mice). The fraction produced alteration of behavior pattern, caused dose-dependent potentiation of pentobarbitone sleeping time and had significant analgesic activity and possesses a potent CNS depressant action. The saline leaf extract of *Bryophyllum calycinum* Salisb produced a dose-dependent prolongation of onset and duration of pentobarbitone-induced hypnosis, reduction of exploratory activities in the head-dip and evasion tests. Moreover, a dose-dependent muscle in-coordination was observed in the inclined screen, traction and climbing tests in mice . The saline leaf extract produced a dose-dependent prolongation of onset and duration of pentobarbitone-induced hypnosis, reduction of exploratory activities in the head-dip and evasion tests and a dose-dependent muscle incoordination in the inclined screen, traction and climbing tests [19, 31, 42].

Anticonvulsant effect:

The CH_2Cl_2/CH_3OH extract reduced seizures induced by pentylenetetrazol, strychnine sulphate and thiosemicarbazide and increases in the latency period of seizures and reduced the duration of seizures induced by the three convulsive agents [31,43-44].

Anti-inflammatory and analgesic effects:

The plant extract significantly inhibited fresh egg albumin-induced acute inflammation and significantly exhibited antinociceptive effects against thermally- and chemically-induced nociceptive pain stimuli in mice[45]. Stigmast-4, 20 (21), 23-trien-3-one, a steroidal derivative obtained from the leaves extract of the plant , also possessed anti-inflammatory effects[31].

The aqueous extract of *Bryophyllum calycinum* leaves were showed antinociceptive, anti-inflammatory and antidiabetic activity. The antinociceptive effect was evaluated by the hot-plate and acetic acid test models of pain in mice. The anti-inflammatory and antidiabetic effects were investigated in rats, using fresh egg albumin-induced pedal (paw) odema, and streptozotocin -induced diabetes mellitus[45].

Effects on renal system:

The aqueous extract of the leaves possessed potent nephroprotective activity in gentamycin-induced nephrotoxicity in rats. The plant hydroalcoholic extract was also found to exert significant diuresis and antiurolithitic activity when given by oral and ip route to rats[39,46-47].

Hepatoprotective effects:

The plant was recorded as hepatoprotectitve . It was significantly lowers the enzyme SGOT, SGPT , SALP and SBLN which increased during liver injury . The juice of the leaves and the ethanolic extract of the marc left after expressing the juice were also found hepatoprotective against CCl4- induced hepatotoxicity. It was also hepatoprotective at histopathological level [48-50]. The juice of the leaves and the ethanolic extract of the marc left after expressing were studied in rats against CCl4-induced hepatotoxicity. It was found that they were effective hepatoprotective as evidenced by *in vitro, in vivo* and histopathological studies. The juice was found to be more effective than the ethanolic extract [12].

Effects on reproductive system:

The plant exerted relaxant effect *in vitro* on the contractility of human myometrium on oxytocin-stimulated contraction at a minimum concentration almost 100-fold lower than in the case of spontaneous contraction [51]. A prospective double-blind trial with orally applied Bryophyllum versus placebo was carried out. Thirty-two patients divided into two groups , 15 patients received Bryophyllum and 17 received the placebo. The time of delivery did not differ between the groups. In both groups the mean time of birth was in the 35 week of gestation. The mean birth weight was slightly higher in the placebo group (2192 g) compared to the Bryophyllum group (1948 g). A transition to the intensive care unit was slightly higher in the placebo group (13) compared to the Bryophyllum group (11) [52].

Gastrointestinal effects:

The methanol-soluble fraction of the leaf extract inhibited the development of a variety of acute ulcers induced in the stomach and duodenum of rats and guinea pigs. Premedication tests in rats revealed that the extract possessed significant protective action against the gastric lesions induced by aspirin, indomethacin, serotonin, reserpine, stress and ethanol. A significant protection with extract was occurred for aspirin-induced ulcer in pylorus-ligated rats and for histamine-induced duodenal lesions in guinea pigs. A significant enhancement of the healing process was also occurred in acetic acid-induced chronic gastric lesions in rats [53].

Cardiovascular effects:

The aqueous and methanolic leaf extracts decreased arterial blood pressures and heart rates of anaesthetized normotensive and hypertensive rats [54]. The effects of aqueous and methanolic leaf extracts of the herb were examined on arterial blood pressures and heart rates of normal (normotensive) and spontaneously hypertensive rats, using invasive and non-invasive techniques. Both the aqueous and methanolic leaf extracts of the plant *(* 50-800 mg/kg iv or ip) produced dose-related, significant (P<0.05 - 0.001) decreases in arterial blood pressures and heart rates of anaesthetized normotensive and hypertensive rats. The hypotensive effects of the leaf extracts were more pronounced in the hypertensive than in normotensive rats. The leaf extracts (0.25 - 5.0 mg/ml) also produced dose-dependent significant (P<0.05 -0.001) decreases in the rate and force of contractions of guinea-pig isolated atria, and inhibited provoked electrical field stimulation (ES-provoked) , as well as potassium and receptor-mediated agonist drugs-induced contractions of the rat isolated thoraxic aortic strips in a non-specific manner. The inhibitory effects of the leaf extracts on the cardiovascular system of the laboratory animals were resistant to physiological doses of standard antagonist drugs [54, 55].

Wound healing:

The ethanolic extract of the leaves of the plant was evaluated for its wound healing activity by using excision wound model in rats. The histological investigation showed that plant leaf ethanolic extract exhibited significant wound healing potential which could be attributed to the presence of steroid glycosides [56].

Antidiabetic effects:

The plant aqueous extract of *B. pinnatum* caused significant reductions in the blood glucose levels of the fasted normal and fasted streptozotocin –treated diabetic rats [12, 45, 57].

Antihistaminic effect:

The methanol extract of the *leaves* has also been reported to have histamine receptor (H1) antagonism in the ileum, peripheral vasculature and bronchial muscle [58].

Contraindications and adverse effects:

In acute toxicity study, it was observed that the LD_{50} values of methanolic extract in mice and rats were 1159.03 and 1459.69 mg/kg respectively and the LD_{50} of aqueous extract were 957.02 and 1064.21 mg/kg respectively. The extracts were found to be non-toxic orally in doses up to 3 g/kg body weight in mice and rats. 2 g/ kg body weight orally for 35 days in rats didn't cause histological changes in kidneys, hearts and spleen. No changes in body weight, hematological and biochemical parameters. There was no death at a maximum acute dose of 5 g kg/ body weight by the oral route. The intraperitoneal LD_{50} was 1.8 g/ kg body weight in rats . Subacute treatment did not significantly alter animal weights, organ-to-body weight ratios, fluid intake, hematological indices and the levels of AST, ALP and albumin. ALT level was significantly reduced (p<0.03) in the treated group. Total bilirubin and conjugated bilirubin levels were not significantly altered in the treated group [12, 59-60].

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