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Review Article

Pharmacognostics Studies And Quality Control of Kalanchoe Pinnata

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ABSTRACT

Kalanchoe pinnata, a succulent plant renowned for its medicinal properties, has garnered significant interest in pharmacognostic studies and quality control assessments. This research explores the phytochemical composition, pharmacological activities, and quality parameters of Kalanchoe pinnata to establish its efficacy and safety for therapeutic use. Standardized methods for identifying key active compounds, such as flavonoids, alkaloids, and phenolic compounds, were employed along with morphological and anatomical evaluations of the plant. Quality control measures, including chromatographic techniques and spectrophotometric analysis, were utilized to ensure the consistency and purity of herbal preparations. The findings highlight the plant's potential in traditional medicine and underscore the importance of rigorous quality control to validate its therapeutic claims. This study contributes to the understanding of Kalanchoe pinnata's role in herbal pharmacotherapy and emphasizes the need for comprehensive quality assessment in herbal medicine.

INTRODUCTION

Numerous plants in Latin America, and Colombia in particular, have been shown to have therapeutic qualities; these plants may contain active compounds with a range of therapeutic uses [1,2]. Among these is Kalanchoe pinnata (Lam.) Pers (syn. Bryophyllum pinnatum, (Lam.) Oken), a species that has been used in traditional medicine to treat bacterial, fungal, and viral infections as well as kidney stones, asthma, inflammatory issues, and ulcers. Its anti-inflammatory [3],

immunosuppressive, and nephroprotective properties (4) Because of its decorative beauty and traditional medicinal qualities, this plant species has substantial economic and medicinal significance. It has been utilized in traditional medicine to treat a number of conditions, including rheumatism, inflammation, immunosuppression, hypertension, kidney stones, liver damage, and anti-tumor properties [5]. The plant can multiply through epiphyllous secondary buds, but as all biotechnological instruments [6] for conservation

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projects require an efficient regeneration system, a technique for quick multiplication through in-vitro culture must be developed. Many succulent plant species are propagated using in-vitro tissue culture, which is thought to be the most effective technique [7, 8]. Alam, et al. [9] explains how *K. tubiflora* was successfully propagated in vitro using leaf explants, how the growth regulators in the culture media were tuned, and how high regeneration rates were attained. Sayari and associates [10]

Taxonomical Classification

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionta (Vascular plants)
 Super division: Spermatophyta (Seed plants)
 Division: Magnoliophyta (Flowering plant)
 Class: Magnoliopsida (Dicotyledonous)
 Subclass: Rosidae
 Order: Saxifragales
 Family: Crassulaceae Stonecrop family
 Genus: Kalanchoe
 Species: *Kalanchoe pinnata* (Lam.) Per^[11]
Synonym: *Bryophyllum calycinum*, *Bryophyllum pinnatum* [12, 13, 14]



Fig. 1 Depiction of *Kalanchoe pinnata* shrub

The plant is a succulent perennial that grows 1-1.5 m tall and has a hollow, four-angled, usually branched stem. The leaves are opposite, decussate, succulent, and 10-20 cm long. The lower leaves are simple, while the upper ones are 3-7 foliate and long-petioled; they are dark green, fleshy, and distinctively scalloped and trimmed in red. The leaf blades are pinnately compound with 3-5 leaflets, 10-30 cm; petiolules 2-4 cm; the leaflet blades are oblong to elliptic x 3-5 cm, margin crenate, each notch bearing a dormant bud capable of developing into a healthy plantlet apex obtuse [12]. The leaves are equipped with rooting vegetative buds, and the inflorescences are terminal panicle, 10-40 cm. pendulous. Calyx tubular, 2-4 cm; Corolla reddish to purple, 5 cm base sparsely ciliate;

lobes ovate-lanceolate; stamens inserted basally on corolla; nectar scales oblong; follicles included in calyx and corolla tube.^[15, 16, 17]

Chemical Constituents

A class of very active compounds known as bufadienolides is found in the leaves. Bufadienolides, such as bryotoxins A, B, and C, have antibacterial, antitumor, cancer-preventive, and insecticidal properties and are structurally and physiologically similar to two additional cardiac glycosides, digoxin and digitoxin [18, 19, 20]. Bufadienolides-
 Bryophyllin A (bryotoxin)^[21]; Bryophyllin B (Fig. 1); Bryophyllol (Fig. 2); Bryophollone (Fig. 3); Bryophollenone (Fig. 4); Bryophynol (Fig. 5)^[22]; Bersaldegennin (Fig. 6).

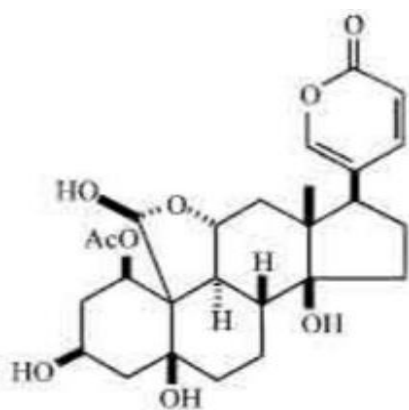


FIG. 1: BRYOPHYLLIN

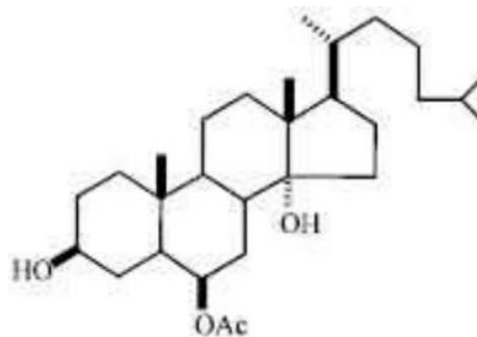


FIG. 2: BRYOPHYLLOL

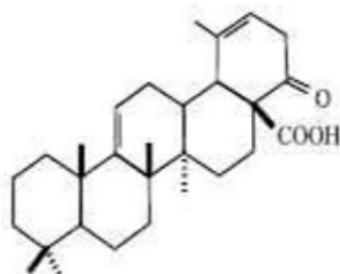


FIG. 3: BRYOPHOLLONE

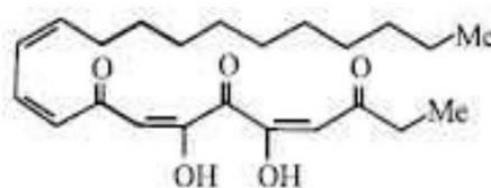


FIG. 4: BRYOPHOLLENONE

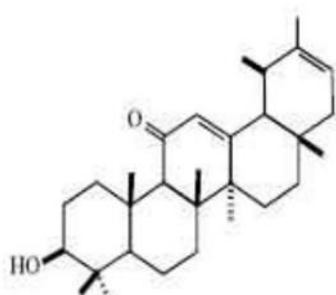


FIG. 5: BRYOPHYNOL

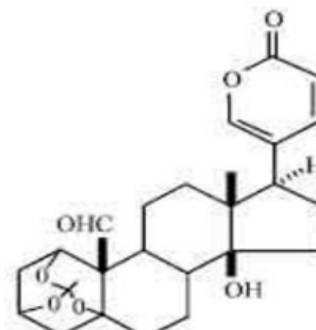


FIG. 6: BERSALDEGENIN

Extract Ion Methods

K.pinnata leaf extracts were produced utilizing three distinct extraction procedures. Conventional Solvent Extraction Plant samples (1 g) were immersed in three different solvent combinations of methanol and water with different ratios (0:100 (T1CSE); 50:50 (T2CSE); 100:0 (T3CSE), volume/volume) for a duration of 24 hours at 60°C. The mixtures were then homogenized at 60°C for 4 hours using a homogenizer (HG-15D, Daihan Scientific Co., Ltd., Seoul, Korea). A basic filter paper assembly was used to remove residue material from solvent, and the samples were then

concentrated using a rotary evaporator at 60°C temperature and 150 rpm (WEV-1001L, Daihan Scientific Co., Ltd., Seoul, Korea). Samples were then freeze-dried and stored at 4°C for additional testing as stated by Truong et al. [23]. Super Critical Fluid Extraction (SFE)

With minor adjustments, the protocol created by Lim et al. [20] for SFE was adhered to. For extraction, the Supercritical Fluid Extractor Model SFT-150 from the USA was utilized. The specific SFE model had a syphonated carbon dioxide cylinder, a volume extractor, and a separator to produce the required solvent pressure for the

process; the assembly also had a syringe pump. In every trial, 200 g of vacuum-dried *K. pinnata* leaf powder was put in the extractor vessel. The weight of the extract was determined at regular intervals during the extraction procedure in order to gauge the extraction rate. Operating parameters were pressure, temperature (40°C), extraction time (two hours), and pressures of 3500 psi (T4SFE), 4500 psi (T5SFE), and 5500 psi (T6SFE). Microwave-assisted extraction following the procedure used by Ghasemzadeh et al. [24] with minor adjustments, several extracts were made by microwave heating at 700-W power for three minutes (T7MAE), six minutes (T8MAE), and nine minutes (T9MAE). Ten milliliters of methanol were mixed with two grams of *K. pinnata* powder. A 50 mL double-necked flask system with a cooling jacket was used to hold the mixture. After that, the mixture in the flask was subjected to 700-W of microwave radiation at various intervals to extract it. Flasks were water-cooled to room temperature once the extraction period was over. Extract filtering using a simple filtration assembly comes next. A rotary evaporator set to 40°C and 150 rpm was used to

remove the solvent in order to concentrate the extract. Extracts were freeze-dried and kept for additional testing. At 20°C.

Isolation And Identification

Identification Of Compound 1: 3-Oxo-Olean-12-ENE: Compound 1 (0.8g) was found to be n-hexane crystals. When vanillin-H₂SO₄ was sprayed over TLC employing an n-hexane: ethyl acetate (90:10) solvent solution with R_f 0.54, it produced a dark purple spot. The existence of a geminal dimethyl group at 1380 cm⁻¹, an olefinic carbon at 1610 cm⁻¹ (C-12), and a carbonyl group at 1706 cm⁻¹ were all identified by IR spectrum analysis. The presence of eight methyl groups was shown by the 8 singlet signal at δ (0.8-1.13) in the ¹H-NMR spectrum. The olefinic proton at H-12 is indicated by a strong signal at δ 5.1 (1H, t). A signal at δ 217.8 in the ¹³C-NMR established the existence of the ketonic functional group. This data is in close accord with the data reported in the literature to be 3-oxo-olean-12-ene (Figure 4) (Lima et al., 2005; Caceres-Castillo et al., 2008). (C-3) also shows the presence of 121.5 & 145.3 ppm, indicating olefinic carbons at C12 & C13, respectively.

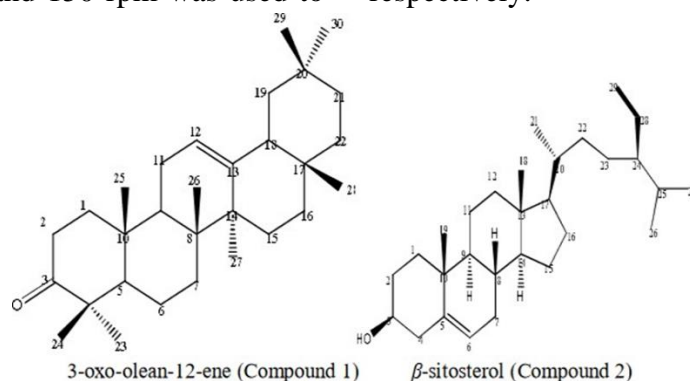


Fig: Chemical Structure of Compound 1 and 2

Identification Of Compound 2: β-Sitosterol

With R_f 0.2, Compound 2 (0.1 g) was separated as a white powder (n-Hexane: CH₂Cl₂ 70:30). After spraying with vanillin-H₂SO₄ using an n-hexane: ethyl acetate (90:10) solvent system, it produced a buff color under UV light that changed to a dark purple spot on TLC. The

existence of a hydroxyl group and an olefinic carbon at 1645.7 cm⁻¹ was indicated by a significant absorption band at 3377.8 cm⁻¹ in the IR spectra of 2 (C-5). The presence of six methyl groups at δ (0.8-1.6) in the ¹H-NMR spectrum indicated the presence of the C-24 ethyl sterol nucleus. Additionally, an oxygen-bearing methine

proton at δ 3.2 (1H, m) and an olefinic proton at δ 5.1 (1H, t). 29 carbons were identified in the ^{13}C -NMR spectra as belonging to the six methyl groups [δ 15.7 (C-18), 17.2 (C-19), and 17.4 (C-21), 18.4 (C-26), 21.5 (C-27), 16.8 (C-29),] vinylic carbon at δ [124.5 (C-5), 121.8 (C-6)], and one oxygenated at δ 79.1 (C-3). The β -sitosterol physical and spectral data (Figure 4) were the same as those reported for compound 2 (Jayaprakasha et al., 2007; Moghaddam et al., 2007).

Qualitative Analysis Of Phytochemical [25]

The screening of qualitative phytochemicals was carried out as follows:

- Ferric chloride test for tannins: About 0.5 g of the extract should be boiled in 20 ml of water, filtered, and a few drops of 0.1% ferric chloride added. Look for indications of blue-black or greenish-brown hue.

The foaming test for saponins involves mixing 5 mg of the extract with 20 ml of distilled water, stirring for 15 minutes, and looking for the production of foam, which is a sign that saponins are present.

- Flavonoids (Lead acetate test): Add a few drops of a 10% lead acetate solution after ingesting 10 mg of the extract. When flavonoids are present, a yellow precipitate forms.

- Terpenoids (Salkowski's test): cautiously add 3 ml of concentrated H_2SO_4 after mixing 5 mg of the extract with 2 ml of chloroform. H_2SO_4 . To determine whether terpenoids are present, look for a reddish-brown interface.

According to Wagner's Test, alkaloids: Apply 1.5% v/v hydrochloric acid to 2 mg of the extract to acidify it. Add Wagner's reagent after filtering. When a brown or reddish precipitate forms, it indicates that alkaloids are present.

- Steroid (Salkowski's test): Mix 2 milliliters of chloroform with 2 milliliters of strong sulfuric acid to dissolve 5 milligrams of the extract. The

presence of steroids is indicated by the emergence of a red color.

- Glycosides (sodium hydroxide test): Dissolve the extract (approximately 5 mg) in 1 ml of water and add 5–6 drops of 10% NaOH. The presence of glycosides is indicated by the formation of a yellow tint.

Pharmacological Activities

Anti-inflammatory activity

Leaf extracts of *Kalanchoe pinnata* were produced in methanol, acetone, chloroform and petroleum ether to investigate its effects on formaldehyde induced oedema on experimental basis. When compared to all other extracts, the methanolic extract had the strongest impact on preventing paw edema. Bradykinin, prostaglandins, serotonin, and histamine were also measured in formaldehyde-induced inflammations from injured cells that have sufficient ability to create the endogenous mediators. These experimental findings thus led to the conclusion that the presence of bufadienolides and other water-soluble extract constituents was primarily responsible for the inhibition of oedema in rats caused by formalin [26]

Anti-microbial activity

Kalanchoe pinnata roots were combined with methanol, chloroform, petroleum ether, and water to create extracts of varying polarity. Because of the presence of diverse flavanoids, these extracts demonstrated a multitude of potential actions when taken orally. These solvent extracts were investigated in murine models of cutaneous leishmaniasis. Quercetin-L-, 3-O-L arabinopyranosyl, 3-

O-L-rhamnopyranoside, and free quercetin dosages per day rhamno pyranoside were administered to the test animals. According to experiments, these substances were able to considerably lower the parasite load and regulate the development of lesions brought on by *Leishmania amazonensis*. The crude *Kalanchoe pinnata* aqueous extract was administered at 320



mg/kg of body weight, demonstrating the efficacy of these flavonoids. According to an HPLC-DAD-MS examination of the extract-treated mice's plasma, the primary metabolites of *Kalanchoe pinnata* quercetin glycosides are quercetin and quercetin glucuronides. These findings demonstrate that quercetin glycosides, which have strong oral activity against cutaneous leishmaniasis, are significant active mechanisms of the aqueous extracts [27].

Anticancer activity

Supert man et al. extracted bufadie no lides from *Kalanchoe pinnata* and tested them for their ability to suppress the early antigen activation of the Epstein Barr virus in Raji cells caused by the tumor promoter. All of the bufadie no lides exhibited good action, but the maximum activity was shown by bryophyllin A [28].

Anticonvulsant activity

Rats were administered *Bryophyllum pinnatum* leaf extract in groups at doses of 50, 100, and 200 mg/kg, and tests were conducted. Muscle tone (Chinney, inclined screen, and climbing tests), anticonvulsant (strychnin and picrotoxin-induced convulsant in mice), and head dip and evasion tests in mice. While 200 mg/kg exhibited the maximum activity, all extracts produced positive outcomes. An aqueous leaf extract of *Bryophyllum pinnatum* at a concentration of up to 20g/kg was found to be cytotoxic [29].

Anti-diabetic activities

Plant hydroalcoholic extract (500 mg/kg body weight) decreases blood glucose, lipid, and low density lipoprotein levels and increases high density lipoprotein levels in both postprandial and streptozosin- induced diabetes [30].

Antifungal activity

In order to assess the antifungal efficacy of Nigerian traditional plants (Vaginal Candidiasis), Adenike A. Ogunshe et al. They compared the plants to several strains of *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida*

pseudotropicalis. They conclude that the ethanolic extract of *Kalanchoe pinnata* does not suppress any of the strains of *C. pseudotropicalis*. Despite having strong inhibitory effects on other species [31].

Antileishmanial activity

Through the use of BALB/c mice and *Leishmania amazonis* (Ima) to cause the disease, Da Silva et al.'s work shows that an aqueous extract of the plant, administered orally, protects mice against increasing infection with Ima. 27 A 30-year-old guy who was voluntarily treated with *Kalanchoe pinnata* after becoming naturally infected with a virulent type of *Leishmania* in Brazil's Amazonia. When he began eating three plant leaves per day for two weeks, the skin lesion was steadily getting bigger. The tumor stopped expanding during this time, and the diameters of the draining lymph nodes reverted to normal. The patient's serum values of urea, creatinin, TGO, and Every TGP was normal. stayed same, indicating that there was no damage to the kidneys, liver, or heart. After the *Kalanchoe* was removed, the lesion began to recur, therefore the patient was treated with traditional pentavalent antimony.[32]

Anti-nociceptive and anti-inflammatory activity

Mice exposed to thermally and chemically produced nociceptive pain stimuli showed notable antinociceptive responses to *Bryophyllum pinnatum* leaf aqueous extracts (BPE, 25-800 mg/kg i.p.). The aqueous extract of plant leaves (BPE, 25–800 mg/kg i.p. or p.o.) dramatically reduced the acute inflammation of the rat hind paw caused by fresh egg albumin.[33]

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