Review Article

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PHARMACOLOGICAL VERSATILITY OF *JUSTICIA PROCUMBENS*: A REVIEW

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ABSTRACT

Justicia procumbens L. a perennial herb belonging to the *Acanthaceae* family, has been used in traditional medicine for centuries to treat various ailments. The plant has been found to possess a wide range of pharmacological properties, including anti-platelet aggregation, anti-retro viral disease (RVD), antiasthmatic, anti-inflammatory, antibacterial, antifungal, antiviral, anti-diabetic, antihelminthic, antiplasmodial, antioxidant and radical scavenging activities. For instance, DW2008, an ethanol extract of *Justicia procumbens*, demonstrated effective anti-asthmatic properties in mouse models. The plant's extracts have also been shown to exhibit cytotoxic effects against various cancer cell lines, including bladder, colorectal, leukemia and liver cancers, indicating its potential as an anti-cancer agent. Additionally, the plant has been found to possess fungicide and insecticide properties, making it a potential natural pesticide. The phytochemical analysis of *Justicia procumbens* has revealed the presence of lignans (primary bioactive compound), flavonoids, alkaloids and phenolic compounds, which are responsible for its diverse pharmacological properties. Anti-platelet aggregation studies reveal that aryl naphthalene lignans from *Justicia procumbens* inhibit platelet aggregation by modulating key signaling pathways, suggesting potential therapeutic applications for thrombotic diseases. This review aims to provide a comprehensive overview of the phytochemical and pharmacological investigations of *Justicia procumbens*, highlighting its potential as a source of novel therapeutic agents for various diseases. Further research is warranted to fully exploit the plant's therapeutic potential and to develop evidence-based remedies for human health.

Keywords: *Justicia procumbens,* Acanthaceae, Aryl naphthalene lignans, Pharmacological properties, Novel therapeutic agents, Traditional medicine

INTRODUCTION

Natural products are recognized as one of the most important sources for the discovery and development of novel medications.[1] Plants have been shown to have enormous therapeutic potential, while many have yet to be studied. A variety of distinct chemical scaffolds with useful pharmacological and therapeutic characteristics can be found in medicinal plants.[2] Numerous chemicals originating from plants have been shown to have antiviral properties; some of these have even been tested in animal research and human clinical trials to determine how effective they are against viruses.[1] From 1981 to 2014, almost 70% of antiviral small molecule medications that were approved were originally derived from natural sources.[3]

Acanthaceae is one of the largest and most diverse flowering plant families, containing 346 genera and 4,300 species. With almost 600 species, *Justicia* is the largest genus in the *Acanthaceae* family and is distributed throughout tropical and pantropical areas. This genus of plants is well known for producing a wide range of secondary metabolite groups, such as lignans, alkaloids, terpenoids, flavonoids and iridoids.[2] Research has demonstrated that *Justicia* species possess a variety of chemical classes, such as vitamins, essential oils, fatty acids (docosanoic acid) and salicylic acid.[4] The entire *Justicia procumbens* L. plant, which is mostly found in Taiwan and South China, has long been used in Chinese folk medicine to cure cancer, pain and fever.[5]

Justicia procumbens L. is a similar variety of popular food and medicine known as "Juechuang." It is extensively distributed in south and southwest China and adapts to tropical and subtropical climates. The entire plant is used as a vegetable to make delectable soups to go with poultry and cattle. Because of its clinical effects of lowering inflammation, eliminating toxic heat and enhancing digestion, it is also being used as a herbal cure for fever, pharyngolaryngeal pain, cancer, asthma, edema, cough, jaundice, urinary tract infection, sore throat, for snake bites and as a fish-killer.[2,6]

The plant *Justicia procumbens* has been reported to contain a number of aryl naphthalide lignans. It was demonstrated that these lignans were cytotoxic to a number of cancer cell lines. In a continued search for novel cytotoxic constituents, three new lignans named Procumbenoside J, Pronaphthalide A and Procumbiene along with a novel natural product, Juspurpudin and twelve known lignans identified as Justicidin A, 5′-methoxy retrochinensin, Rostellulin A, Syringaresinol, Justicidin B, 6′-hydroxyl justicidin A, 6′-hydroxyl justicidin B, Diphyllin, Tuberculatin, Taiwanin C, Justicidin C and Pinoresinol by comparison of their NMR spectral data with the published literature data were isolated from an ethanol extract of the entire plant of *Justicia procumbens*.[5]

A new lignan glycoside called procumbenoside H and a new cyclopeptide alkaloid called justicianenes A were identified from the aerial portion of the plant *Justicia procumbens* as part of an ongoing hunt for novel cytotoxic components.[7] From the EtOH extract of the entire plant of *Justicia procumbens* L., three new 14-membered cyclopeptide alkaloids, named justicianenes B, C and D were isolated. Their structures were established using comprehensive NMR spectroscopic data and the absolute

stereochemistry of the ring-bonded a-amino acids in the cyclopeptide alkaloids was established using ECD (electronic circular dichroism) spectra.[8]

Botanical Description

The perennial herb *Justicia procumbens* L. is slender, frequently tufted, prostrate or ascending and branching. The length of the stems varies from 10 to 40 cm. The leaves are entirely or partially crenate at the margin, elliptic to oblong-ovate or ovate, 7–20 mm long, 5–20 mm broad, obtuse at both ends, glabrous or sparsely hairy and have 1.5–3 cm long petioles. The pink, 6-7 mm long blooms are carried in terminal, extremely thick, cylindrical spikes that measure 1–5 cm in length and roughly 5 mm in diameter.

Figure 1: *Justicia procumbens* aperennial herb

The calyx is 3–4 mm long, 4–partite deep; the stamens are 2; they are tied to the upper part of the corolla; the anthers are unevenly spaced; the ovary tip and style base are hairy; the sepals and bracts are green, linear–lanceolate and hairy; it is 4-6 mm thick, outdoor hairy, uneven, circular, sub-acute or acute segments. The glabrous, slightly hairy fruit (capsule) is about 4 mm long, with orbicular, brown, striate seeds that measure 1 x 1 mm. Throughout all of India, water willow grows up to 1500 meters above sea level.[9]

Pharmacological Properties

Anti-platelet aggregation activity

The efficacious crude extracts derived from the entire plant of *Justicia procumbens* was evaluated using an *in vitro* assay for anti-platelet aggregation. Following the incubation of *Justicia procumbens* extracts with apheresis platelets, gene chips were used to identify regulatory genes and high affinity chemicals were identified by HPLC-MS. Computational docking technique was used to examine the effective components and putative target proteins. An *in vivo* anti-thrombotic test was also used to assess the chemical with the strongest expected activity. Arylnaphthalene lignan aglycones containing one methylenedioxy group and two methoxy groups of *Justicia procumbens* are useful components for inhibiting platelet aggregation, according to an experimental study. The Gq-PLC-PKC and Gi-PI3K-MAPK signaling pathways are inhibited by these substances through the suppression of gene expression in ITGB3, PRKCA, PIK3CG and MAPK14. The characteristics of Chinese medicine's multicomponent and multitarget synergistic treatment were represented in these outcomes. The results of this study show that *Justicia procumbens* has potential anti-platelet aggregation activity. Further *in vivo* animal activity studies of *Justicia procumbens* are essential for the confirmation of anti-platelet aggregation action.[10]

Anti-Retro Viral Disease activity

In anti-HIV activity, from *Justicia procumbens*, twenty-one lignans including three novel ones were isolated. One of the spectroscopic methods used to discover the chemical structures of the new lignans was 1D and 2D NMR analysis. These substances' cytotoxic and anti-HIV properties were assessed. A secure and effective "One-Stone-Two-Birds" assay procedure was used to measure their antiviral efficacy against the HIV virus. This protocol uses H5N1 HA generated HIV virions and VSV-G pseudo typed HIV virions in a parallel assay. In addition to the new lignans procumbenoside N, procumbenoside O and secoisolariciresinol dimethyl ether acetate, 18 known compounds were identified by directly comparing their spectroscopic data with those published in the literature. These compounds were identified as procumbenoside A, procumbenoside B, procumbenoside E, ciliatoside B, ciliatoside A, diphyllin, cilinaphthalide A, justicidin E, justicidin D, justicidin C, secoisolariciresinol dimethyl ether, 5-methoxy-4, 4′-di-O-methylsecolariciresinol, secoisolariciresinol, hemiariensin, ariensin, secoisolariciresinol dimethyl ether diacetate, hinokinin and 5'-methoxy-4'-O-methyllariciresinol, respectively. The novel secoisolariciresinol dimethyl ether acetate demonstrated anti-HIV-1 activity, as evidenced by its IC_{50} value of 5.27 μmol•L−1 and selective index (SI) value of 2.2. Procumbenoside A and diphyllin, two aryl naphthalene lignans that are well-known, both showed inhibitory action against HIV-1, with IC_{50} values of 4.95 (SI>6.2) and 0.38 μmol•L−1 (SI=5.3), respectively. Therefore, the findings suggest that *Justicia procumbens* have antiviral activity.[11]

Cytotoxic activity

Justicia procumbens was used to isolate three new lignans, Pronaphthalide A, Procumbiene and Procumbenoside J as well as a novel natural product called Juspurpudin and twelve others known lignans. Comprehensive spectroscopic analyses were used to clarify the structures of the novel compounds and the data from three of them shed light on the conformational equilibria that are present in them. With the exception of compound 2, all compounds were assessed for their *in vitro* cytotoxic activities against Human LoVo and BGC-823 cell lines. It was discovered that eight of them possessed strong cytotoxicity. The findings from the structure-activity relationship (SAR) analysis indicated that the essential element was the parent structure of 2-carbonyl aryl naphthalide lactone connected with 6- and 7-OMe, the polarity of substituent's on C-4 may have a significant impact on the activity, An appropriate cyclic lipophilic group at apiofuranose's C-3" and C-5" on C-4 may improve activity, which would maximize the application of (Procumbenoside J) in a manner similar to VP-16 (ETOPOSIDE).[5]

Analysis of potential antitumor active component

A sensitive and reliable LC-ESI-MS/MS method was developed and validated for the detection of 6'-hydroxy justicidin A (HJA), a putative anticancer active component isolated from *Justicia procumbens* in rat plasma, utilizing a simple liquid-liquid extraction (LLE) sample preparation method. Chromatographic separation was performed on an Agilent Zorbax-C18 column (2.1 mm × 50 mm, 3.5 m) utilizing a step gradient program with the mobile phase of 0.1% formic acid aqueous solution and acetonitrile with 0.1% formic acid. HJA and Internal standard (buspirone) were identified with electro spray positive ionization mass spectrometry in the multiple reaction monitoring (MRM) modes. This approach had good linearity and showed no endogenous interference with the active chemical or internal standard peaks. The LLOQ for HJA in 50μl of rat plasma was 0.50 ng/ml. The proposed and validated method was effectively used to quantify and characterize the pharmacokinetics of HJA in rats following intravenous and oral administration of 0.25 mg/kg HJA. The oral bioavailability (F) of HJA was determined to be 36.0 ± 13.4%, with an elimination half-life (t1/2) of 1.04 ± 0.20 hours.[12]

Bladder cancer activity

6'-hydroxy justicidin A (HJA), a recently discovered substance derived from *Justiciaprocumbens*, uses a caspase-dependent mechanism to cause human bladder cancer EJ cells to undergo apoptosis. HJA has been demonstrated in bladder cancer activity work to promote apoptosis in human bladder cancer EJ cells. ROS production was necessary for the caspase activation action. The ROS and caspase-3, caspase-8, and caspase-9 inhibitors can prevent apoptosis in EJ cells. Using MTT and SRB, the cytotoxicity activity was measured. Laser scanning confocal microscopy was utilized to visualize and quantify intracellular ROS. Using a propidium iodide (PI) apoptosis detection kit and flow cytometry, apoptosis was quantified. Using the GloMax luminescence detector and the Caspase-Glo 3, 8, 9 test kits, the activation of caspases (caspase-3, caspase-8, and caspase-9) was assessed in turn. Under JC-1 dye microscopy, loss of mitochondrial membrane potential was seen. Using quantitative real-time PCR analysis, the expression of a protein linked to cell death was found. By reducing cell proliferation, decreasing SOD activity, increasing the level of reactive oxygen species (ROS), and inducing apoptosis, HJA significantly suppressed the growth of human bladder cancer EJ cells. It was suggested by the activation of caspase-8, caspase-9, and caspase-3 that HJA might be triggering both intrinsic and extrinsic apoptotic pathways. This extract was unsuccessful due to suppression of caspase-3, caspase-8, and caspase-9; hence, caspase is required for 6'-hydroxy justicidin Ainduced apoptosis. Additionally, HJA deregulated the expressions of p53 and Bax in EJ cells and interfered with the mitochondrial membrane potential ($\Delta\psi_m$).[13]

Colorectal cancer activity

Justicidin A (JA), a pure chemical derived from *Justicia procumbens*, is shown in a study on colorectal cancer to inhibit AKT/mTOR and activate type III PI3K/beclin 1 signaling pathways, which in turn cause autophagy of human colorectal cancer cells. By using immunoblotting and confocal microscopy, treatment with JA not only enhanced the expression of the autophagic marker microtubule-associated protein 1 light chain-II, but it also enhanced the production of acidic vesicular organelles in HT-29 cells as shown by flow cytometry. Subsequent investigation revealed that in JA-treated cells, the expression of type III PI3K, becline 1 and Atg5-Arg12 was up whereas that of p-AKT, p-mTOR, and p-p70S6k was lowered. Using flow cytometry and propidium iodine labeling after Annexin V-FITC staining, it was possible to see that early apoptosis was induced 12 hours after autophagy. Notably, pre-treating the cells with the autophagy inhibitor 3-methyladenine lowered the early apoptotic rate generated by JA. Oral administration of JA (6.2 mg/kg) once a day for 56 days was observed to reduce the proliferation and trigger apoptosis and autophagy of HT-29 cells transplanted to NOD-SCID mice, indicating that JA has chemotherapeutic potential on human colorectal cancer cells.[14]

Antileukemia activity

In human leukemia K562 cells, 6'-hydroxyjusticidin B causes a critical imbalance in Ca^{2+} homeostasis and mitochondria-dependent cell death. 6'-hydroxyjusticidin B (HJB), a substance that was isolated from *Justicia procumbens*, shows encouraging biological characteristics. We looked at the pharmacokinetic characteristics of HJB in rats as well as its mechanism of action on human leukemia K562 cells in our Antileukemia study. The outcomes showed that HJB induced apoptosis and markedly reduced K562 cell growth. In addition, HJB led to an increase in cytosolic calcium and a decrease in the mitochondrial membrane potential deltaPSIm. It also elevated the amount of the calcium homeostasis regulator protein TRPC6. Following HJB treatment, there was a considerable rise in the expression of p53, the activity of caspase-8 and caspase-9. HJB also exhibits a relatively long elimination $t_{1/2}$ and a rapid absorption rate, suggesting a longer *in vivo* residence period. The findings show that HJB altered the function of mitochondria and calcium homeostasis to trigger the p53 signaling pathway, which in turn reduced the growth of K562 cells and promoted apoptosis. According to the pharmacokinetic analysis, HJB has a moderate metabolism and is well absorbed *in vivo*. These findings suggest HJB as a possible cutting-edge substitute for established human leukemia treatments.[15]

Liver cancer activity

In human hepatoma cells, caspase-8 plays a crucial upstream executor role for mitochondria during justicidin A-induced apoptosis. Isolated and refined from a methanolic extract of *Justicia procumbens*, justicidin A has been shown to inhibit the growth of many tumor cell lines, hepatoma cells, and TNF-α release *in vitro*. Given that both caspase-8 and mitochondria are impacted, the study's findings indicate that justicidin A triggers both intrinsic and extrinsic apoptotic pathways in hepatocellular carcinoma (Hep 3B and Hep G2 cells). DNA fragmentation, phosphatidylserine externalization, and sub-G1 cell accumulation are the hallmarks of apoptosis induction. The release of cytochrome c and second mitochondria-derived activator of caspase/direct IAP binding protein with low pI (Smac/DIABLO) from mitochondria activates caspase-9 and caspase-3. Activation of caspase-8 increases the amount of tBid to change mitochondrial membrane potential ($\Delta\psi_m$). Further encouraging the apoptotic process in mitochondria is the rise in Bax and Bak and the fall in Bcl-xL. *In vivo*, justicidin A's cytotoxicity is equally potent. ZIETD, a caspase-8 inhibitor, lessened the disruption of $\Delta\psi_m$ caused by justicidin A. Oral justicidin A (20 mg/kg/day) significantly inhibited the growth of Hep 3B transplanted in NOD-SCID mice. These findings suggest that caspase-8 mediates the justicidin A-induced apoptosis in these cells, which is thereafter succeeded by mitochondrial disruption.[16]

Muscle atrophy

Skeletal muscle atrophy is linked to a variety of conditions, including cancer, inflammatory diseases, neuromuscular disorders, and acute critical illness. *Justicia procumbens* L. has been utilized as an herbal medicine, although the pharmacological impact of *Justicia procumbens* on muscular atrophy has yet to be reported. Here, we examine how n-butanol fraction of *Justicia procumbens* (JPBuFr) in C2C12 myotubes inhibits the dexamethasone (DEX)-induced atrophy of the muscles. Under a fluorescent microscope, the diameter of the myotubes, the myosin heavy chain (MHC) positive area, the generation of reactive oxygen species (ROS), and the contents of the mitochondria were measured. Western blots were used to investigate different proteins associated with synthesis or degradation. JPBuFr considerably reduced the reduction of myotube diameter, mitochondrial content, ATP level, myosin heavy chain, and myogenin expression caused by DEX. Furthermore, when DEX and JPBuFr were co-treated, phosphorylation of Akt, mTOR, and p70S6K proteins increased while reactive oxygen species generation and expression of protein degradation factors (MuRF1, Atrogin-1, and FoxO3a) reduced compared to DEX alone. These findings indicate that JPBuFr may have potential protective benefits against muscle atrophy, making it a candidate for the creation of anti-atrophic health functional foods.[17]

Anti-asthmatic activity

Dong-Wha Pharmaceutical, Inc., Co. produces DW2008 (justicidin A and B), an anhydrous ethanol extract of *Justicia procumbens*, as a potential anti-asthmatic medication. The results of anti-asthmatic activity showed that DW2008, as opposed to a decoction or ethanol extract of *Justicia procumbens*, selectively

decreased T helper 2 (Th2) cytokines in mouse splenocytes and ameliorated ovalbumin-induced airway inflammation by down regulating pulmonary infiltration of differential inflammatory cells and Th2 cytokines in a mouse asthma model. DW2008 also considerably decreased the thickness of the airway epithelium and suppressed hyper responsiveness of the airways. The main peaks of DW2008 (justicidin A and B) were higher than those of the other extracts, according to HPLC analysis. Justicidin A and B demonstrated a protective effect against ovalbumin-induced airway inflammation and dramatically reduced Th2 cytokine levels in mice spleen cells. Our results imply that DW2008 has potential as an anti-asthmatic drug since it successfully reduces allergic airway inflammatory reactions and airway hyper responsiveness in a mouse model of asthma.[18]

Antibacterial and antifungal activity

Three fractions were obtained from the dried and powdered *Justicia procumbens* plant material: aqueous, ethanolic, and chloroform. Using the disk diffusion method and the broth dilution method, the antimicrobial activity was assessed against the following pathogen species: *Bacillus subtilis, Streptococcus pyogens, Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Shigella flexneri, Proteus vulgaris* (Gram negative), and one fungal species *Candida albicans*. As positive controls, 10µg of ketoconazole and 10µg of ciprofloxacin were utilized. It was noted that against tested gram-negative bacteria, the aqueous, ethanolic, and chloroform extracts of *Justicia procumbens* demonstrated strong antibacterial activity. The antimicrobial activity of ethanol and chloroform extracts against gram-positive bacteria was moderate. For every studied organism, the ethanol extract shown stronger antibacterial activity than the chloroform extract. The yeast *Candida albicans* was not inhibited by the ethanol or aqueous extracts. However, there was a noticeable inhibitory effect of chloroform extract against *Candida albicans*. It can be inferred from the study's findings that the Siddha medical system has traditionally used *Justicia procumbens* for its antibacterial properties.[19]

Antiviral activity

One of the structural distinguishing features of aryl naphthalene lignans (ANLs) is their propensity for atropoisomerism, which may result in bioactivity differences. However, stable ANL atropisomers are rare in nature. We extracted nine ANL glycosides from *Justicia procumbens* during the phytochemical investigation, and four of them were shown to be novel stable atropisomers. The study of the circular dichroism (CD) and electronic circular dichroism (ECD) data allowed for the determination of their absolute configurations. The antiviral potential of ANL compounds was assessed as entry inhibitors against H5N1 influenza virus, vesicular stomatitis virus (VSV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Of these compounds, compound number five was found to be the most potent, with IC50 values ranging from 0.0063–1.13 µM. The lack of notable antiviral activity in the atropisomers suggests that the antiviral activity of ANL compounds may be largely attributed to a free rotation of the biphenyl aryl-aryl bond.[20]

Anti-diabetic and anti-helminthic activity

The entire *Justicia procumbens* plant was collected, dried, and ground into a powder. Studies on pharmacognostic factors such as moisture content, extractive value, and proportion of foreign matter were conducted. It was determined through organoleptic evaluation what color, smell, texture, and taste were. The leaves and stem were identified through microscopical examinations. The identification of powder characteristics was done using powder microscopy. The drug substance in powder form was extracted using a series of solvents, including ether, ethyl acetate, acetone, methanol, and water. For additional phytochemical and biological research, extract was employed. Studies evaluating phytochemicals reveal phytoconstituents such as proteins, flavonoids, alkaloids, phenolic compounds, and saponins, among others. To find the *in vitro* antidiabetic and antihelminthic actions, more research was done.[21]

***In-vitro* antidiabetic activity (Alpha amylase inhibition technique)**

At scheduled intervals, 250µl of 1% starch solution is added in 0.02M sodium phosphate buffer, PH 6.9. For ten minutes, incubate the reaction mixture at 25°C. 500µl of the Di-nitro salicylic acid color reagent will stop the reaction. Following a 5-minute incubation period in a bath of boiling water, the test tubes are cooled to room temperature. Measure the absorbance at 540 nm after diluting the reaction mixture with 5 milliliters of distilled water. Acarbose was used as a reference at different concentrations (12.5 - 100µg/ml). Three duplicates of each experiment were conducted, with a parallel setup serving as the control. The findings were computed using the following formula to express the inhibition as a percentage.

$$\text{Inhibitory activity (\%)} = (Ac\text{-}As/Ac) \times 100$$

Where, as is the test substance's absorbance and Ac is the control's absorbance.

***In-vitro* anti-helminthic activity (Assessing antihelminthic efficacy with earthworms)**

Earthworms, with an average length of 6 cm, were put in Petri dishes with 10 milliliters of methanol and 15 milliliters or 25 milliliters of ethyl acetate extract. The standard medicine utilized was albendazole solution, while the control was pure water. Following an incubation period at 37°C, the worms' motility was assessed. This was carried out after the worms were given free reign to roam around in the washbasin and the contents of the Petri dishes were poured in. The living worms displayed motion when the end of each worm was tapped with the index finger and slightly compressed, whereas the dead worms exhibited no motility. The incubation procedure was repeated when the motile worms were placed back into their corresponding Petri dishes with medication solution. Similar to the results previously published, the worms in the control group were alive for a minimum of 12 days. After confirming that the worms did not move when shaken violently or when submerged in warm water (50°C), the duration of paralysis, any type of motility activity, and the time until death were examined and recorded. Both tests reveal that methanolic extract had the highest activity when compared to the conventional medication. Overall, it has been established that the entire *Justicia procumbens* plant studied possesses good antidiabetic and antihelminthic efficacy. Additional investigation of *Justicia procumbens* on *in-vivo* study is necessary.[21]

Anti-inflammatory activity

A 95% alcohol extract of *Justicia procumbens* was tested for *in vivo* anti-inflammatory efficacy in albino rats. It had promising anti-inflammatory efficacy at a dose of 100 mg/kg body weight. Plethysmographic measurements of oedema caused by a sub-plantar injection of formalin in the rat's hind paw are used to determine anti-inflammatory activity. The highest activity was found during the fourth hour following treatment. The findings are highly significant ($p<0.001$) and equivalent to conventional phenylbutazone. The presence of steroids and flavonoids in the alcohol (95%) extract of *Justicia procumbens* may be the cause of its anti-inflammatory properties, as suggested by the extract's initial phytochemical testing.[22]

Fungicide and insecticide activity

Methanol, chloroform, acetone, ethyl acetate, and hexane extracts were produced from *Justicia procumbens* by combining the methods of cold-soaking and ultrasonic extraction in order to demonstrate the fungicidal and insecticidal activity of the extracts. Using five species of plant pathogenic fungus, *Colletotrichum gloeosprioides, Phomopsis asparagi, Pyricularia grisea, Fusarium graminearum,* and *Botrytis cinerea*, the inhibitory effects of the extract were investigated using the inhibition zone method. The toxicity of methanol extract was tested against four plant pathogenic fungus and bacteria species (*C. gloeosprioides, P. asparagi, B. cinerea,* and *Xanthomonas campestris*), as well as three insect species (*Aedes albopictus, Musca domestica, and Pieris rapae*). Extracts of *Justicia procumbens* at a concentration of 8 mg/ml in various polar solvents inhibited five plant pathogenic fungi. Methanol extract exhibited over 50% inhibition rates against *B. cinerea, P. asparagi,* and *C. gloeosprioides. Justicia procumbens* methanol extracts shown superior fungicidal and insecticidal action. The EC_{50} of methanol extract was 5.94 mg/mL−1, 4.61 mg/mL−1, and 5.27 mg/mL−1 for *C. gloeosprioides, P. asparagi, and B. cinerea*, respectively, against their mycelium growth. The respective matching EC_{90} values were 63.69 mg•mL−1, 58.01 mg•mL−1, and 54.57 mg•mL−1. When tested at 0.25~1.00 mg•mL−1, methanol extract shown potent inhibitory action against X. campestris. At 0.125 mg•mL−1, the resulting extract exhibited a modest level of inhibitory action. The MIC of the methanol extract was 0.0625 mg•mL−1. *A. albopictus, M. domestica, and P. rapae* were the targets of the methanol extract LC_{50}, which were 0.1958 mg•mL−1, 0.3514 mg•mL−1, and 0.2877 mg•mL−1, respectively. The respective equivalent EC_{95} values were 3.053 2 mg•mL−1, 2.5844 mg•mL−1, and 0.9884 mg•mL−1. This implied that extracts from *Justicia procumbens* would have greater potential for use as a biological insecticide in farming.[23]

Antiplasmodial, radical scavenging and antioxidant activity

Twenty plants belonging to the Acanthaceae family had their crude extracts (CH_2Cl_2 and MeOH) tested for antiplasmodial, antioxidant, and radical scavenging properties. With IC_{50} values ranging from 10 to100 µg/ml, the MeOH extract of *Justicia procumbens* demonstrated the most antiplasmodial action. The findings suggest that the majority of the plant's active antiplasmodial component in *Justicia procumbens* is nonpolar. *Justicia procumbens* CH_2Cl_2 extracts had a 56% inhibition rate when it came to scavenging DPPH free radicals. It should be mentioned that the majority of the active ingredient suggested that the molecules scavenging radicals were somewhat polar. Crude MeOH extract of *Justicia procumbens* demonstrated antioxidant activity with an ORAC unit of 3.1, according to the ORAC assay. The fact that the *Justicia procumbens* plant's action was limited to the MeOH extract suggests that the component causing it was relatively polar. According to our observations, substances exhibiting ORAC assay-based antioxidant activity invariably include phenolic hydroxy groups, which are essential to the activity.[24]

CONCLUSION

The development of contemporary civilization has been significantly influenced by herbal medicine. Various research studies have demonstrated the long-standing traditional therapeutic use of *Justicia procumbens*, an intriguing example of a plant with numerous applications. Traditionally *Justicia procumbens* is used in asthma, cough, edema, fever, jaundice, urinary tract infection, sore throat, snake bites and many more. *Justicia procumbens* and their active extracts contain major pharmacological and medicinally important phytochemical components such as lignans that exhibits several pharmacological properties such as anti-platelet aggregation activity, anti-Retro Viral Disease (anti-HIV), anti-asthmatic (anti-allergic), muscle atrophy, cytotoxic (anti-cancer or antitumor), anti-inflammatory, antibacterial, antifungal, antiviral, anti-diabetic, antihelminthic, antiplasmodial, antioxidant, radical scavenging, fungicide and insecticide activities. Further exploration of additional research work on *Justicia procumbens* is needed for futuristic study to eradicate the disease and improve the quality of life. This review concludes that the plant *Justicia procumbens* shows therapeutic benefits and can be useful to generate a variety of commercial pharmaceuticals.

ABBREVIATIONS

NMR: Nuclear Magnetic Resonance
EtOH: Ethanol extract
CD: Circular dichroism
ECD: Electronic circular dichroism
HPLC-MS: High-performance liquid chromatography-mass spectrometry
PLC: Phospholipase C
PKC: Protein Kinase C
PI3K: Phosphatidylinositol 3-kinase
ITGB3: integrin beta chain beta 3
PRKCA: protein kinase C alpha
PIK3CG: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform
MAPK14: Mitogen-activated protein kinase
LC-ESI-MS: Liquid Chromatography-Electrospray Ionization-Mass Spectrometry
MTT: (3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
SRB: Sulforhodamine B
PCR: Polymerase Chain Reaction
SOD: Superoxide dismutase
Bax: Bcl-2-associated protein x
Bak: Bcl-2 homologues antagonist/killer
LLOQ: Lower limit of quantitation
AKT: AK strain transforming (Protein Kinase B)
mTOR: The mammalian target of rapamycin
p-p70s6k: 70-kDa ribosomal protein S6 kinase
Atg5: Autophagy protein 5
FITC: Fluorescein Isothiocyante
HT-29: Human colorectal adenocarcinoma cell line
NOD-SCID: No obese diabetic/severe combined immunodeficiency
Bcl-xL: B-cell lymphoma-extra large
ANLs: Aryl naphthalene lignans
ORAC: Oxygen Radical Absorbance Capacity
MeOH: Methanol extract
VSV: Vesicular stomatitis virus
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
DPPH: 2, 2-diphenyl-1-picrylhydrazyl

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