Original Article

Evaluation Of Antioxidant And Anti-Microbial Activities Of Kalanchoe Pinnata And Pongamia Pinnata

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Abstract

The aim of this study was to investigate and determine the antioxidant and antimicrobial activities of hydroalcoholic extracts of *K. pinnata* (leaves) and the seeds of *P. pinnata*. The antioxidant activity was evaluated using the 2,2-diphenyl1-picrylhydrazyl radical, while the antibacterial activity was assessed using the disc diffusion in-vitro assay method. The hydroalcoholic extracts of *K. pinnata* and *P. pinnata* seeds exhibited significant antioxidant activity, with IC50 values of 69.93µg/ml and 99.06µg/ml, respectively. The antimicrobial activity of the extracts was evaluated against Gram-positive (B. subtilis) and Gram-negative (K. pneumoniae) bacterial strains. The results showed that the hydroalcoholic extracts of *K. pinnata* and *P. pinnata* seeds contain potential antimicrobial activity due to the presence of secondary metabolites. These findings indicate that the hydroalcoholic extracts of *K. pinnata* leaves and *P. pinnata* seeds possess strong antibacterial and antioxidant properties, making them promising sources of natural compounds for the development of new drugs.

Keywords: antioxidant, antibacterial, antimicrobial compounds, flavonoids, phenolic acids, ascorbic acid.

INTRODUCTION

The discovery and development of new medications rely heavily on natural compounds derived from medicinal plants [Mercy et al., 2018]. According to Ebong et al. (2015), over 25% of medications contain substances derived from higher plants. The World Health Organization estimates that between 65 and 80 percent of the world's population, who reside in developing nations, rely mostly on plants for basic healthcare [Johnson et al., 2015]. The antibacterial and antioxidant properties of medicinal plants have attracted a lot of interest lately [Al-Rifai et al., 2017]. Antioxidant capabilities of medicinal plants have been studied [Nandhakumar et al., 2013]. According literature research work have been found a number of phenolic components such flavonoids and phenolic acids, ascorbic acid and vitamin E, are primarily responsible for the antioxidant activity [Sawadogo et al., 2006]. These organic antioxidants work wonders to stop the harmful effects of oxidative stress brought on by free radicals [Gruz et al., 2011]. Reactive oxygen species (ROS), also known as free radicals, have been linked to the pathophysiology of numerous diseases, including ageing, cancer, coronary artery disease, hypertension, diabetes, and neurological disorders [Santharam et al., 2015; Ferguson et al., 2010]. On the other hand, today's germs are becoming more and more resistant to the existing synthetic and semi-synthetic antibacterial drugs [Stankovic et al., 2016]. The available antibiotics also result in a number of negative medication responses, including immunosuppressant and hypersensitivity [Tsuruga et al., 2007]. The pharmaceutical industry urgently needs to produce newer antimicrobial medicines that are effective against germs and less hazardous to the body because to these negative effects and the ongoing development of bacterial resistance. Medicinal herbs are one of the most major natural sources of antibacterial compounds [Kusuma et al., 2014]. Finding novel natural sources of safe and affordable antioxidants and antibacterial chemicals is therefore crucial. *Pongamia pinnata*, also referred to as karanja locally, is one of the more popular medicinal plants. It is a member of the Fabaceae family of mangrove plants. It grows in the littoral areas of south-east Asia, Australia, and Fiji. It is a medium-sized glabrous tree with a short bole that can reach heights of around 18 metres [Simin et al., 2002]. The seeds are useful in the treatment of hypertension, bronchitis, whooping cough, skin conditions, and rheumatoid arthritis. The bark is traditionally used in piles. The leaves are effective as therapeutic baths and for rheumatic aches. Roots can be used to treat ulcers, gum disease, and gonorrhoea [Rastogi et al., 2001; Chauhan et al., 2002]. It is also used in the treatment of diabetes and used as an anti-inflammatory, antiplasmodial, anti-noneceptive, anti-hyperglycemic, anti-lipodoxidative, anti-diarrheal, anti-ulcer, anti-hyper ammonic, and antioxidant substance in ayurvedic and unani medicine [Sagwan et al., 2010]. A member of the Crassulaceae family, *Kalonche pinnata* is a succulent plant also known as Patharchata in Hindi. *K. pinnata* is used pharmacologically for a variety of conditions, including treating cancer patients' sleep issues [Simoes-Wüst et al., 2015]. *K. pinnata*, sometimes known as the "wonder of life," is used to heal infections in the feet of diabetics (Cawich et al., 2014). When exposed to UV-B light, the plant

produces wound periderm in its leaves. This tissue shields the plant from the stress state [Nascimento et al., 2015]. Because of its remarkable insulin secretagogue action, the dichloromethane fraction of the steam distillate of *K. pinnata* leaves can be utilised to treat diabetes [Patil et al., 2013]. *K. pinnata* is also effective for issues with sleep. The findings inspire additional clinical research on sleep-related issues [Lambrigger-Steiner et al., 2014]. In mouse models, the aqueous leaf extract of *K. pinnata* showed antihistaminic and expectorant action [Salami et al., 2013]. To our knowledge, no systematic research has been done on the *in-vitro* antioxidant activity of medlar leaf extract, and earlier studies have paid less attention to the leaf extract's antibacterial activities. Therefore we were select both of plant for investigation and assessment of antioxidant and antimicrobial activities of hydroalcohalic extract using leaf of *K. pinnata* and seed of *P. pinnata* part.

MATERIALS AND METHODS

Plant material

In the month of January 2018, Bhopal (M.P.) locals collected the leaf of *K. pinnata* and the seed of *P. pinnata*. Reagents and all the chemicals were used analytical-grade.

Extraction

K. pinnata leaf and *P. pinnata* seed were cleaned, shade-dried, and coarsely powdered. The coarsely powdered material and was used to prepare the hydroalcoholic extract using a Soxhlet apparatus for 48 hours. After the completion of the extraction process, the extract was filtered and evaporated. The percentage yields of the dried extracts were calculated, and suitable storage conditions were established for further study [Mehta et al., 2018, Mahto et al., 2022, Tiwari et al., 2020, Shahnawaz et al., 2019, Pandey et al., 2016].

Qualitative phytochemical analysis of plant extract

The preliminary phytochemical analysis of *K. pinnata* and *P. pinnata* extracts was performed using standard procedures [Shukla et al., 2016, Gupta et al., 2015, Sharma et al., 2013, Bajaj et al., 2012].

Antioxidant activity by DPPH assay

Antioxidant activity of K. pinnata and P. pinnata seed was determined using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay method. K. pinnata and P. pinnata seed extract test sample were prepared at different dilution, and were added 0.9 mL of 100 mmol/L Tris-HCl buffer (pH 7.4) and 1 mL of DPPH (500 mmol/L in ethanol). Prepared different strength of test sample of plant extract and was mix well then set aside for 30 minutes. UV-absorption of test samples was taken at 517nm using UV-visible spectrophotometer [Charde et al., 2011, Garg et al., 2018, Mahto et al., 2022]. The reaction mixture without DPPH was used for background correction. The antioxidant activity was calculated using the following equation:

Percentage antioxidant activity of extract/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Antimicrobial activity using well diffusion method

Three different species of bacteria, including two gram-positive bacteria (*B. subtilis*) and one gram-negative bacteria (*K. pneumonia*), were tested using the well diffusion method. The standard medication employed was ciprofloxacin. The bacterial dilutions were made using the 0.5 McFarland turbidity standards and streaked separately on various agar plates. Müller-Hinton agar (Oxoid, UK) was produced, poured onto several sterile petri dishes, and then mixed with the turbidity standards. A negative control against the bacterial cell lines was DMSO. Aseptic methods were used to create wells in the agar plates so that the *K. pinnata* and *P. pinnata* plant extract could be added. The petri dishes were then incubated for 24 hours at 37 degrees Celsius before the zone of inhibition was measured [Khandelwal et al., 2005, Kokate et al., 1994, Bhandarkara et al., 2015, Vikram et al., 2019].

Statistical analysis

Data were presented as the mean and standard deviation of three samples (SD).

RESULTS AND DISCUSSION

Extraction yields

The extraction yield is influenced by the solvents, extraction duration, temperature, and chemical makeup of the sample. Extraction yield of *K. pinnata* and *P. pinnata* hydroalcohalic extract was determined. Percentage yield of hydroalcoholic extracts of *Kalanchoe pinnata* leaf and *Pongamia pinnata* seed was found to be 4.8 and 3.7 respectively.

Phytochemical test

Results

Phytochemical test was performed and was found to be flavonoid, saponins, diterpenes, Proteins and amino acids present in *K. pinnata* and *P. pinnata* hydroalcohalic extract respectively. The phytochemical results were indicated that *K. pinnata* and *P. pinnata* hydroalcohalic extract is having polyphenolic secondardy metabolites. These chemical compositions and phenolic secondary plant metabolites, like Alkaloid, flavonoids and carotenoids, may have antioxidant and antibacterial capabilities and supported our hypothesis behind the selection of plant.

Antioxidant activity

An agent that prevents the consumption of oxygen is referred to as an antioxidant. Antioxidants act as metal chelating agents, enzyme inhibitors, electron givers, hydrogen donors, peroxide decomposers, radical scavengers, and hydrogen donors. Antioxidants also act as protectors of our body against a polluted environment or free radicals. They can be produced by either ex-situ processes such as smoking, radiation, pollution, and medication or in-situ processes such as regular cell metabolism. Oxidative stress occurs when an excess of free radicals cannot be eliminated. Oxidative stress can cause many ailments, including autoimmune disorders, cancer, aging, cardiovascular and neurological diseases. However, the body combats oxidative stress by a variety of processes that are created naturally on-site or ex-situ through food or supplements [Tanaka et al., 2002, Stankovic et al., 2016, Tiwari et al., 2020].

The antioxidant activity is explained by the availability of antioxidants such as phenolic compounds and flavonoids, which are effective oxygen radical scavengers. The redox characteristics of phenolic compounds are mostly attributed to their ability to act as reduction agents, hydrogen donors, and singlet oxygen quenchers. The findings were reported in units of moles of vitamin C per gram of extract, and various quantities of vitamin C were produced for the calibration curve [Kusuma et al., 2014].

Ascorbic acid was used as a reference, and its IC50 value for inhibiting the DPPH radical was found to be 17.68 µg/mL, while the hydroalcoholic extract of *K. pinnata* leaf and *P. pinnata* seed displayed an IC50 value for antioxidant activity, indicating its ability to inhibit 50% of the DPPH radica. The result is shown in Table 4 and figure 1.

Table 4: Antioxidant activity of Hydroalcohalic extract of *Kalanchoe pinnata* and *Pongamia pinnata*.

Antioxidant activity (µmol Vitamin C\g)		
Ascorbic acid IC50	***Kalanchoe pinnata* IC50**	***Pongamia pinnata* IC50**
17±.68µg/ml	60±.93µg/ml	99±.06µg/ml

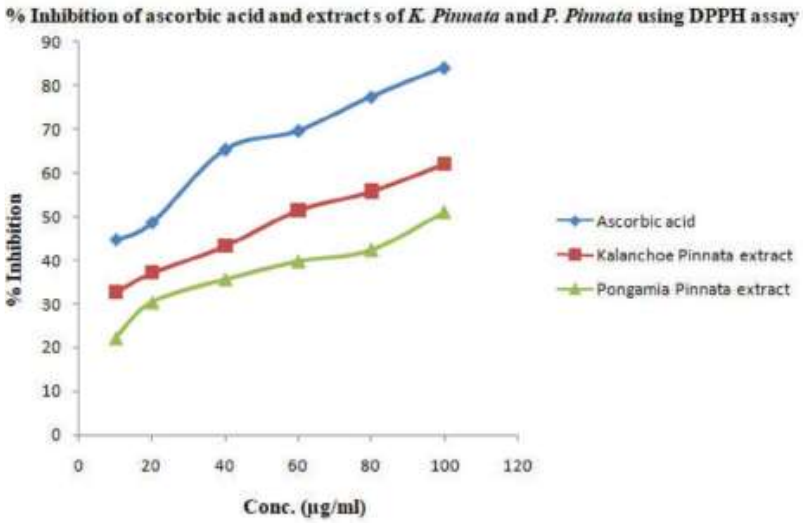

Figure 1: Inhibition (%) of DPPH against extract concentration of Hydroalcohalic extract of *Kalanchoe pinnata* and *Pongamia pinnata*

Antimicrobial activity

A well diffusion method was used to evaluate the antibacterial properties of the hydroalcoholic extract of *Kalanchoe pinnata* and *Pongamia pinnata*. The hydroalcoholic extracts of *Kalanchoe pinnata* and *Pongamia pinnata* showed excellent antibacterial activity as like standard drug against *B. subtilis* and *K. pneumoniae*, respectively, with inhibition zones of 10 mm and 14 mm. The results are shown in Table 5, 6 and Figure 1, 2.

Table 5: Antimicrobial activity of standard drug against selected microbes

S.N.	**Name of drug**	**Microbes**	**Zone of inhibition**		
			30µg/ml	20 µg/ml	10 µg/ml
	Ciprofloxacin	*B. subtilis*	20±0.15	17±0.74	12±0.5
		K. pneumonia	36±1.69	28±1.24	19±4.71

Figure 1: Antimicrobial activity of standard drug against selected microbes

Table 6: Antimicrobial activity of Hydroalcoholic extract against selected microbes

S.N.	Name of drug	Microbes	Zone of inhibition		
			100µg/ml	50µg/ml	25µg/ml
	Kalanchoe pinnata	*B. subtilis*	11±0.57	10±0.81	8±0.94
		K. pneumonia	8±0.74	6±0.24	6±0.1
	Pongamia pinnata	*B. subtilis*	12±0.86	8±0.57	6±0.2
		K. pneumonia	10±0.94	7±0.5	6±0.1

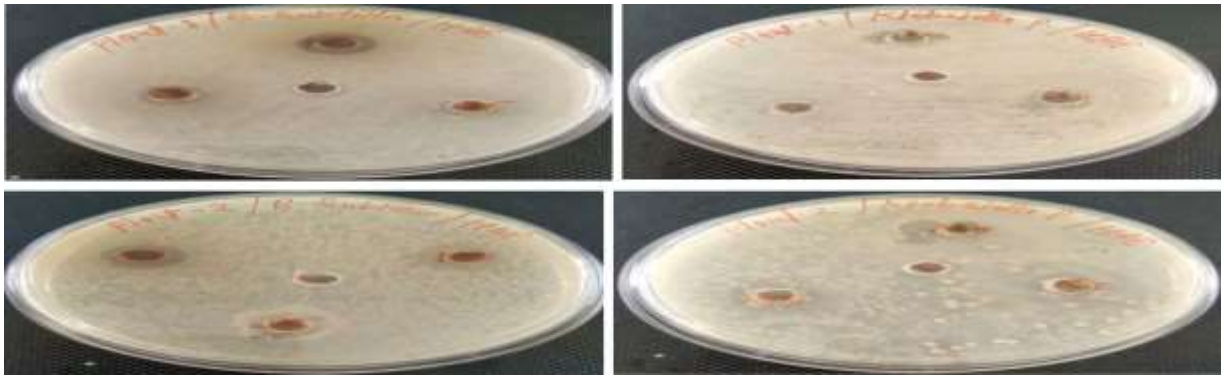

Figure 2: Antimicrobial activity of Hydroalcoholic extract against selected microbes

DISCUSSION

The hydroalcoholic extracts of *Kalanchoe pinnata* and *Pongamia pinnata* were tested for their total phenolic content, flavonoid content, and antioxidant activity. The results indicate that the extracts contain significant amounts of medicinally active compounds. Moreover, the plants were found to possess good antibacterial and antioxidant properties. Strong antimicrobial activity has been observed against the gram-positive bacteria *B. subtilis* and gram-negative bacteria *K. pneumonia*. This finding demonstrates the extract's selectivity for both types of bacteria [Santharam et al., 2015, Vikram et al., 2019]. In this experiment, various concentrations of hydroalcoholic extracts of *Kalanchoe pinnata* and *Pongamia pinnata* were used in the well diffusion method. Further research is needed to determine the potential of using these extracts as antibacterial agents. However, the results showed that both plants' hydroalcoholic extracts had significant inhibitory effects against pathogenic bacteria. Given the increasing resistance of pathogenic bacteria to various antibiotics and antimicrobial drugs, there is a need for new drug discoveries. The hydroalcoholic extracts of both *Kalanchoe pinnata* and *Pongamia pinnata* demonstrated strong antibacterial properties [Mishra et al., 2019, Bhandarkara et al., 2015, Kusuma et al., 2014].

CONCLUSION

Nowadays, researchers are exploring the use of traditional medicine to develop new pharmaceuticals for treating various disorders. *Kalanchoe pinnata* and *Pongamia pinnata* hydroalcoholic extracts have been used in traditional medicine for many years to treat skin infections and other ailments. However, serious issues such as microorganisms developing antibiotic resistance have emerged in recent times, leading to the need for novel medications. Our studies and data analysis on the hydroalcoholic extract of *Kalanchoe pinnata* and *Pongamia pinnata* plants indicate that the plants can be used as antioxidant, antibacterial, and anticancer agents. The conclusion of this study provides new insights into the antioxidant and antibacterial properties of *Pongamia pinnata* and *Kalanchoe pinnata*. Further research is recommended to explore the potential antioxidant and antibacterial properties of their hydroalcoholic extracts. Both plant extracts have shown promising antibacterial activity against both gram-positive and gram-negative bacteria.

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