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EVALUATION OF THE PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF Kalanchoe pinnata AGAINST PLANT PATHOGENS ISOLATED FROM DISEASED PLANT PATHOGENS

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ABSTRACT
The antimicrobial activity of Kalanchoe pinnata (Syn Bryophyllum pinnatum) against clinical pathogen is well documented in literature but there is paucity of information on its effect against plant pathogens. This work attempts to evaluate inhibitory activity of Kalanchoe pinnata (Syn Bryophyllum pinnatum) on selected plant pathogens. Aqueous, acetone, ethanol and methanol leaf, stem and root extracts of Kalanchoe pinnata (Syn Bryophyllum pinnatum) were prepared using standard techniques. Extracts were tested against bacteria and fungi isolated from some diseased plants, both singly and in combination with standard antimicrobials. Inhibitory activity was determined using agar well diffusion technique as well as broth dilution technique. Results indicate that the leaf ethanol extract was most effective against the plant pathogens. Zones of inhibition (mm) ranged from [19.0 ± 0.32] for Aspergillus flavus to [23.5 ± 0.22] for Xanthomonas campestris. Meanwhile, the Minimum inhibitory concentration (MIC) reduced from 6.25 mg/mL to 3.13 mg/mL (for Xanthomonas oryzae and Xanthomonas campestris) when leaf extract was used in combination with streptomycin. Furthermore, MIC reduced from 1.56 mg/mL to 0.78 mg/mL (for Aspergillus flavus and Aspergillus niger) when extract was used in combination with cycloheximide. Ethanol leaf extract of Kalanchoe pinnata (Syn Bryophyllum pinnatum) was most effective against selected plant pathogens. Also, effectiveness of extract was enhanced when used in combination with regular antimicrobial. Kalanchoe pinnata (Syn Bryophyllum pinnatum) may become useful as a biological control agent for plant disease pathogens.

Keywords: Kalanchoe pinnata, plant pathogen, biological control agent.

INTRODUCTION

Kalanchoe pinnata (Syn Bryophyllum pinnatum) belongs to the Crassulacea family and it's usually brought up as life plant, love plant, miracle leaf, cathedral bells and Geothe plant. It's cosmopolitan in Hawaii, China, Australia, Madagascar, America and tropical Africa. It's found largely within the South-Western a part of Federal Republic of Nigeria and brought up as "Karan Mascallachi" or "Shuka Halinka" in Hausa (Yahaya et al., 2015).

K. pinnata may be a succulent plant, 3-5 feet tall, 3.2cm wide, tall hollow stems, fleshy dark inexperienced leaves that are rough distinctively, trimmed in red and bell-like nodding flowers (Imaobong et al., 2020). Between the teeth of their margin, accidental buds seem ensuing to the assembly of rots stems and leaves. The miniature plant life shaped on the margin of its leaves, falls to the bottom, rot and become larger plants (Azuonwu et al., 2017).

World Health Organization defines medicative plant as any plant that contains substance in its organs which will be use for the synthesis of medication (WHO, 1997). Medicative plants like K. pinnata carries with it phytochemical with curative properties like phenanthrene, tannins, taraxasterol, saponins, triterpenes, steroids, alkaloids, aurones, phenolic resin acid, organic acid, oil flavonoids and alternative chemicals that are biologically active (Akinnibosun and Edionwe, 2015).

In ancient medication, the leaves juice are used for excretory organ stones treatment, application against headache, cardiovascular disease, cancer, inflammation and as a well-liked remedy for fever (Akinnibosun and Edionwe, 2015). Medicative plants like K. pinnata, are effective against microorganism and zymosis in man or skin like wound infections, infection and dandruff. The human skin outer layer is consistently at risk of micro-organisms attack. Herbal extract are quickly rising as natural antimicrobial additives or preservatives in high demand (Cox et al., 2010).

Besides the final anti-pyretic, medicine activity of K. pinnata, bryophillin C isolated show robust insecticidal activity against silkworm larvae (Supratman et al., 2001). Its phytochemical analysis known some bufadienode compounds like bryophillinA, bersaldegenin-3-acetate and bryophillin C with insecticidal properties. Plant-based pesticide represents a huge untapped supply.

In Nigeria, the plant is especially legendary for its therapeutic uses and effective antimicrobial activity against human pathogens. However, the aim of this analysis is to judge the antimicrobial activity of the plant extract against plant pathogens isolated from pathological plant components.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh K. pinnata leaves were collected from totally different locations of the Delta state university Site III, Nigeria. The leaves of K. pinnata obtained were known by plant biologist within the department of botany, Delta state university and identification was confirmed fittingly with literature (Owhe-Ureghe et al., 2012; Ilondu et al., 2020). The leaves obtained were dry, ground into a fine powder using standard laboratory mortar and pestle. It absolutely was hold on in a very sterile air-tight container to forestall contamination.

PREPARATION OF PLANT EXTRACT

Fifty (50) g of dried macerated leaf powder was dissolved in 500ml of H2O to create an aqueous liquid extract (Akinnibosun and Edionwe, 2015). The answer was allowed to face 24 h and centrifuged at 3000rpm. The pure extract obtained was filtered using Whatman paper and filtrate was gaseous to xerotes at 100oC using steam water-bath. Ethanol, methyl alcohol and solvent extract were ready using an equivalent procedure with fermentation alcohol, methyl alcohol and solvent severally as solvents. The extract was hold on in refrigeration at 4oC.

ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF PATHOGENS FROM PATHOLOGICAL DISEASED PLANT PARTS

The fungus and microorganism isolates employed in the current study had been isolated from pathological plants: Telfana occidentalis, Vernonia amydalina, Carica papaya, Manihot esculenta and Solanum lycopersium. The pathological plants were obtained from the premises of the Delta State University Site III and cultured aseptically into nutrient agar, Potato Dextrose Agar (PDA) and Sabouraud Dextrose agar (SDA) for the growth of bacteria and fungi respectively.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATES.

Visible microbial growth was observed after the incubation period. The isolates were characterized and identify based on cultural, morphological and biochemical characteristics (Prescott et al., 2008; Hussain et al., 2010).

DETECTION AND QUANTIFICATION OF PLANT PHYTOCHEMICALS

a. Detection of Alkaloids

Mayer's Test: Plants extract were dissolved in each hydrochloric acid and filtered. The filtrates were appropriately treated with Mayer's reagent. The Yellow cream precipitate formed indicated the presence of alkaloid.

b. Detection of Cardiac Glycosides.

Killer-Killiani's Test: A mixure of 40 ml glacial acetic acid, 1 drop of 2.0 % FeCl3 and 1 ml of H2SO4 was added to 10 ml of plant extracts. The formation of a brown ring between the layers confirmed the presence of cardiac glycosides.

c. Detection of Tannins

Ten (10) ml each of bromine water was added to 0.5 g K. pinnataleaf extracts. The discoloration of bromine water confirmed the presence of tannins.

d. Detection of Flavonoids

Shinode Test: Plant extracts were mixed with magnesium stripand hydrochloric acid. The formation of pink color indicated the presence of flavonoids.

e. Detection of Saponins

Five (5.0) ml each of distilled water was added to the plant extract to form a broth.Fewdropsofoliveoil were mixed with the broth formed the formation of foams indicated the presence of saponins.

f. Test for Steriods

Five (5.0) ml plant crude extract was prepared. 2.0 ml concentrated H2SO4 and chloroform were added to the plant extract. The appearance of red color in the lower chloroform layer indicated the presence of steroids.

g. Detection of Reducing Sugars (De-Oxy Sugars)

To discover the presence of reducing sugars 0.5g of plant extract was pulverized. 20ml of distilled water was added to the pulverized plant extracts and filtered accurately. 1.0 ml of alkaline copper reagent was added to 1.0ml of the filtrates. The mixture was boiled at100oC for 5min and allowed to cool down. 2.0ml of distilled and 1.0ml of phosphomolybdic acid reagent was added (Prescott et al., 2008; Ordonez et al., 2006). Spectrophotometric analysis was carried out and the absorbance read at 420nm.

QUANTIFICATION OF PLANT PHYTOCHEMICALS

a. Total Determination of Flavonoids

The aluminium chloride quantitative analysis methodology was utilized in the determination of total flavonoids within the extract (Ordonez et al., 2006). The 0.5 ml of 2% AlCl3 solution was added to 0.5 ml of sample solution. The absorbance was measured at 420 nm using a spectrophotometer after 1 h at room temperature. Yellow flavonoids confirmed the presence of flavonoid and total flavonoid was calculated as quercetin equivalent (mg/g). The calibration curve range from 10-100 mg/ml.

b. Determination of Total Saponins

Hundred (100) cm3 of 20% aqueous ethanol were added to 20g of the plant. The sample was heated for 4h at 550c, stirring incessantly. The mixture obtained was filtered and the residue was re-extracted with 200 ml volume of 20% ethanol. Reduction of the extract to 40 ml over a waterbath at about 90oC was carried out. 20ml volume of diethyl ether was added to the concentrate and vibrated vigorously. 60 ml of n-butanol was added to the aqueous layer recovered for its purification. The n-butanol extracts were washed doubly with 10 ml volume of 5% aqueous sodium chloride and heated in a water bath. The sample was dried in the oven to a standard weight after evaporation and the saponin content was calculated (Obadoni and Ochuko,2002).

c. Determination of Total Tannins

Five hundred (500) mg of the plant extract was measured and 50 ml of distilled water was added. The mixture was jolted in a mechanical shaker for 1h and filtered. 5ml filtrate obtained was mixed with 2.0 ml volume of 0.1m FeCl3 in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm using the spectrophotometer for 10min (Akinnibosunet al., 2008).

d. Determination of Total Alkaloid

Total alkaloids content was analyzed according to the standard method described by Imaobonget al. (2020). 5 g of the plant extracts was measured and 200 ml of 10 % acetic acid in ethanol was added. The mixture was allowed to stand for 4 h aseptically. The solution was filtered and concentrated in a waterbath until 1/4 of the original volume was achieved. Few drops of concentrated ammonium hydroxide were added to the concentrated extract to obtain a complete precipitation. The precipitate obtained was washed with dilute ammonium hydroxide. The mixture was filtered, the residue was dried and weighed.

e. Determination of Total Steroids

Plants extract of1.0ml volume was measured. Sulphuric acid (4N, 2 ml) and iron (iii)chloride (0.5% w/v, 2 ml) were added to 1.0 of plant extract, followed by the addition of potassium hexacyanoferrate (iii) solution (0.5% w/v, 0.5 ml). The mixture obtained was heated at 720C for 30 min a water bath and stirring occasionally. The mixture was diluted to a 10 ml volume in a 10 ml volumetric flask and absorbance was measured at 780nm (Imaobonget al., 2020; Anjooet al., 2005).

f. Quantitative Examination of Cardiac Glycosides

Cardiac glycosides of the plant extract were determinedaccording to Igoliet al. (2005).10% of plant extract were mixed with 10ml Baljet's reagent(Baljet's reagent: 95 ml of 1%picric acid and 5% 0f 10% NaOH). The mixture was allowed to stand for 1h. The mixture was mixed with 20 ml distilled water for dilut6ion. The absorbance was measure at 49 nm.

g. Examination of Reducing Quantitative Sugar Content (Rsc)

The quantitative examination of reducing content was carried out using 3,5-dinitro salicylic acid (DNSA)method (Azuonwuet al.,2017). DNSA reagent was prepared by dissolving 30g of sodium potassium tartaric acid 1g of DNSA in 80ml of 0.5N NaOH at 450C. The solution was cooled to 250 C and diluted with 100ml distilled water. 2ml volume of DNSA reagent was added to 1 ml of plant extract in a test tube and stored at 950C for 5 mins. The mixture was cooled and diluted by adding 7 ml of distilled water. The absorbance of the mixture was read at 540 nm.

STANDARDIZATION OF ISOLATES

The muddiness of microorganism and fungous suspensions was adjusted using the McFarland normal as a reference. All of the check microbial isolates were cultured in Mueller Hinton broth, followed by the matching of the microorganism and fungous suspensions to the muddiness similar to 0.5 McFarland resolution (1-2 x 108cfu/ml). Totally different extract concentrations (10, 20, 40, 60, and eighty mg/ml) were prepared and hold on in properly corked test tubes.

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF EXTRACT ON ISOLATES (AGAR WELL DIFFUSION TECHNIQUES)

Mueller-Hinton Agar (Oxoid, UK) and Sabourand Dextrose Agar plates were prepared and 0.1 ml volume of each diluted microbial isolates was transferred into the agar plates aseptically (Anjoo et al., 2005). A sterile Hockey stick was used to aseptically spread evenly the inoculum. The inoculated agar plates were kept for 30mins for the isolate to diffuse appropriately into the medium. 5mm sterile cork borer was used to bore wells on the medium.0.1ml volume of the K. pinnata extract was dropped in the wells. The agar plates were labeled accordingly.

At the equivalent time, the control was placed in a separate well.The inoculated agar plates were incubated properly and viewed for the diameter of inhibition zones. The diameter of inhibition zones was suggested by clear areas without growth around the well.

RESULTS, DISCUSSION AND CONCLUSION

Phytochemical Screening

The results of the qualitative phytochemical screening of acetone, aqueous, ethanol and methanol of leaf, stem and root extracts of K. pinnata as illustrated in Table 1. The tables revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannin and reducing sugar in both plant parts. However, cardiac glycosides, steroids and tannins were not detected in the aqueous leaf extracts. Alkaloids, cardiac glycosides, flavonoids, saponins, steroids and tannins were detected in the ethanol leaf extract.

The results of the quantitative phytochemical screening of leaf ethanol extracts of K. pinnata in Table 2 shows that the concentration of alkaloids was 1.25±0.09mg/g. The results of cardiac glycosides concentrations in leaf extracts were 3.15±0.10 mg/g.Flavonoid contents in the leaf ethanol extract was 3.11 ±0.23mg/g while the saponins concentration were 2.21±0.21 mg/g. Steroids and tannins concentration in the leaf ethanol extracts was 2.30±0.22 mg/g and 1.18 ±0.06 mg/g respectively. The reducing sugar contents detected was 3.05 ±0.15 mg/g.

Microbial resistance to many antibiotics is turning into a supply of challenge and concern to public health. Given the increasing rate of antimicrobial drug resistance ravaging not solely the African continent however the globe at giant. Alternative, effective and affordable substitutes are essential if bacterial infections are to be properly controlled.

Phytochemicals are secondary metabolites of plants legendary to exhibit various medicine and organic chemistry effects on living organisms. several plants containing alkaloids and flavonoids have diuretic drug, medicine and analgesic effects. Alkaloids are capable of reducing headaches related to cardiovascular disease. It's been rumored that alkaloids are often employed in the management of cold, fever and chronic inflammation.

Flavonoids are legendary for inhibitor activity and thence assist to shield the body against cancer and alternative chronic diseases (Jindal et al., 2012). Tannins are legendary to exhibit antiviral, medicine and antitumour activities. glucoside is employed as hypercholesteremia, hyperglycemia, inhibitor, anticancer, medicine and weight loss. The presence of those phytochemicals in K. pinnata employed in this study (Tables 1-3) supports their use as medicative plants.

These chemical constituents could be responsible for their antibacterial activity (Etimet al., 2016). Different plant parts contain a complex of chemicals with distinctive biological activity (Kayode and Kayode, 2011), that is assumed to flow from to toxins and secondary metabolites, which act as attractants or deterrents (Ilondu et al., 2020). Over the years, these bioactive principles have been exploited in tradomedical follow for the treatment of assorted ailments (Alabi et al., 2005).

ANTIMICROBIAL ACTIVITY

Table 3 shows the result of the antibacterial and antifungal activity of leaf ethanol extracts of K. pinnatarespectively tested against four fungi strains and two bacterial strains at 12.25mg/mL concentrations. Streptomycin and cycloheximide were used as the standard. The concentrations used were 1 mg/mL for both standards. The extracts showed strong antimicrobial activity against test organisms. Results from Table 3 showed that A. niger, A. flavus, C. henningsii, C. cassiicola, X. oryzae and X. campestris were susceptible to ethanol leaf extract of K. pinnata at 12.25mg/mL concentrations.

The zone of inhibition of X. oryzae was 21.8 ±0.32mm while that of X. campestris was 23.5 ±0.22mm. The zone of inhibition of the ethanol leaf extracts against fungal pathogens was 19.6 ±0.20mm, 19.0±0.32mm, 21.1± 0.22mm and 20.6±0.45mm for A. niger, A. flavus, C. henningsii, C. cassiicola respectively. Higher antimicrobial activity was observed against C. henningsii (21.1± 0.22mm) and X. campestris (23.5± 0.22mm) for fungal and bacterial plant pathogens.

Minimum restrictive Concentrations (MIC) Of Extract And Extract With standard Antimicrobials

MIC of extracts and extract with standard antimicrobial was determined as the lowest extract concentration that showed the largest inhibition zone. The MIC of leaf extract was observed to be higher against A. niger(1.56 mg/mL), A. flavus (1.56 mg/mL), C. henningsii (3.13 mg/mL), C. cassiicola (3.13 mg/mL), X. oryzae (6.25 mg/mL) and X. campestris (6.25 mg/mL) while the reverse was the case for the extract with standard antimicrobial.This implies the activity of synergy in the inhibition of plant pathogens.

Antimicrobial resistance of pathogenic bacteria to current synthetic drugs has necessitated the investigation into new, safe, efficient, and cost-effective antimicrobial agents as an alternative for controlling infectious diseases (Khan et al., 2012). The extent of sensitivity of the test organisms to the plant fractions was assessed by activity the zone of inhibition after 24h incubation. Table 4 shows the antimicrobial activity of K. pinnata leaf extracts using different extracting solvents.

The results disclosed that the ethanol extract of K. pinnata was effective against the test organisms than the other extracting solvents. X. campestris showed the highest susceptibility to K. pinnata ethanol extract, while A. flavusshowed the least susceptibility. This can be in agreement with the observations of Anmara et al. (2009)

Table 4 shows MIC of K. Pinnata ethanol leaf extracts and synergy. This explains the rationale for the very best antimicrobial activity of the synergy using ethanol as the extracting medium. The ethanol leaf extract had a lower impact on the test organisms, compared to the leaf extract and standard antimicrobial synergy. This showed that each acted synergistically against the test isolates (Adwan et al., 2010).

The results of this synergism are supported by Dawoud et al.(2013). The additive and synergistic impacts of phytochemicals increased the antimicrobial effect of the synergism extract (combined) (Matchimuthu et al., 2008). According to Cain et al. (2003) synergistic activity suggests diiferent mode of action of the combining components. The synergism thus has shown potential antimicrobial impact against the test organisms and may thus, be employed in the treatment of infections caused by the test organisms.

Ethanol leaf extract of Kalanchoe pinnata (Syn Bryophyllum pinnatum) was most effective against selected plant pathogens. Also, effectiveness of extract was enhanced when used in combination with regular antimicrobial. Kalanchoe pinnata (Syn Bryophyllum pinnatum) may become useful as a biological control agent for plant disease pathogens.

Table 1 Phytochemicals present in various parts of the plant according to the solvent used

Constituents	Leaf			Stem				Root			
	AC	AQ	ET	MET	AC	AQ	ET	MET	AC	AQ	ET	MET
Alkaloid	+	+	+	-	+	+	+	-	+	+	+	-
Cardiac glycoside	+	+	-	+	+	+	-	+	-	+	-	+
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	+	+	+	+	+	+
Steroid	+	-	+	+	-	-	+	+	-	-	+	+
Tannin	-	-	+	+	-	-	+	+	-	-	+	+
Reducing sugar		+	+	+	+	+	-	-	+	+	+	+

Extracts - AC: acetone; AQ: aqueous; ET: ethanol; MET: methanol

Table 2 Concentration of phytochemicals in the leaf ethanol extract

Constituents	Concentration (mg/g)
Alkaloid	1.25 ± 0.09
Cardiac glycoside	3.15 ± 0.10
Flavonoid	3.11 ± 0.23
Saponin	2.21 ± 0.21
Steroid	2.30 ± 0.22
Tannin	1.18 ± 0.06
Reducing sugar	3.05 ± 0.15

Table 3 Inhibitory activity of ethanol leaf extract on diseased plants isolate

Plant pathogens	Zones of inhibition (mm)		
	Ethanol leaf extract (1 mg/mL)	Streptomycin (12.25mg/mL)	Cycloheximide (1 mg/mL)
Aspergillusniger	19.6 ± 0.20	-	20.2 ± 0.20
Aspergillusflavus	19.0 ± 0.32	-	21.0 ± 0.25
Cercosporidiumhenningsii	21.1 ± 0.22	-	22.6 ± 0.22
Corynesporacassiicola	20.6 ± 0.45	-	23.1 ± 0.10
Xanthomonasoryzae	21.8 ± 0.32	23.5 ± 0.23	-
Xanthomonascampestris	23.5 ± 0.22	23.8 ± 0.10	-

Table 4 Minimum inhibitory concentration (MIC) of extract and extract with standard antimicrobial

Plant pathogens	MIC (mg/mL)		
	Leaf extract only	Streptomycin With extract	Cycloheximide With extract
Aspergillusniger	1.56	-	0.78
Aspergillusflavus	1.56	-	0.78
Cercosporidiumhenningsii	3.13	-	1.56
Corynesporacassiicola	3.13	-	1.56
Xanthomonasoryzae	6.25	3.13	-
Xanthomonascampestris	6.25	3.13	-

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