

PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF THE FRESH AND DRIED WHOLE PLANT EXTRACTS FROM *Commelina Benghalensis*.

EVALUACION FITOQUIMICA Y DE LA ACTIVIDAD ANTIMICROBIANA DE LOS EXTRACTOS DE LA PLANTA SECA Y FRESCA DE *Commelina Benghalensis*

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Abstract

Commelina benghalensis has been reported in folkloric medicine to poses antimicrobial, anti-inflammatory and abortifacient properties. However, neither phytochemical nor antimicrobial evaluation of this plant has been reported in literature. The extracts from shade dried plant material yielded greater percentages of extractives from the same amount of plant material than the extracts from freshly collected plant material. Dry plant material produces 4,35% for ethanol extract and 5.53% for aqueous extract > that from the fresh plant material; 1,20% for ethanol extract and 1,24% for aqueous extract. The phytochemical screen led to the discovery that *Commelina benghalensis* contains 10 pharmacologically active chemical compounds and all these were present in the ethanol extracts while only 5 of these were present in the aqueous extracts thus rendering the ethanol extracts more pharmacologically active than the aqueous extracts. The ethanol extracts are superior in performance to the aqueous extracts. In both ethanolic extracts, activity against *Candida albicans* > *Escherichia coli* > *Staphylococcus aureus*. The ethanol extracts showed relatively comparable activities to gentamycin and nystatin which are locally available standard antimicrobial agents on the market. Aqueous extracts showed virtually no activity against the *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* strains.

Key words: antimicrobial activity, *Commelina benghalensis*, Uganda, Africa.

Resumen

Commelina benghalensis es una planta herbácea que para la medicina tradicional posee propiedades antimicrobianas, anti-inflamatorias y abortifacientes. No obstante, no se tienen informes en la literatura de evaluaciones sobre el efecto antimicrobiano de sus extractos. Se evaluaron extractos de etanol y agua de la planta total, fresca y secada a la sombra. Los extractos de la planta seca aportaron los rendimientos de 4,35% para el alcohol y 5,53% para el agua y la planta fresca 1,20% para el alcohol y 1,24 para el agua. El tamizaje fitoquímico demuestra la posible presencia de 10 grupos diferentes de productos naturales para los extractos de alcohol y 5 para los de agua. Los extractos de alcohol por lo tanto tienen más posibilidades de poseer

actividad biológica de interés. Los extractos de alcohol son mas activos como antimicrobianos siendo el orden de actividad *Candida albicans* > *Escherichia coli* > *Staphylococcus aureus*. La actividad es comparable a gentamicina y nystatin utilizados como controles positivos. Los extractos acuosos no mostraron actividad frente a los tres microorganismos evaluados.

Palabras clave: efecto antimicrobiano, *Commelina benghalensis*, Uganda, Africa.

Introduction

In an attempt to preserve traditional medicine knowledge and its use for finding active chemical structures for medicine, it is necessary to have co-operative efforts between modern and traditional health workers and researchers (HAMILL *et al.*, 2000, 2003). In Uganda, as in other developing countries, traditional medicine occupies a central place among rural communities but enough information is not available about the chemical composition and real biological possibilities of most of the plants traditionally in use (TABUTI, 2003).

Commelina benghalensis is an annual or perennial herb with fleshy creeping stems that root daily at the nodes and grows as a weed in tropical and subtropical countries including eastern, western, central and southern Africa. In Uganda, *Commelina benghalensis* in the District of Kalangala is used by the Baganda by pounding the whole plant fresh, as with a mortar and pestle, or crushed and packed into the skin or over a wound as a wound ecboic (HAMILL *et al.*, 2000, 2003). *Commelina benghalensis* has a minimal level of phytochemical or biological activity reported with seemingly corroborative results; *Commelina benghalensis* L. has been collected from the wild in Bulamogi and used to treat insanity by boiling the leaves in meat (TABUTI *et al.*, 2003).

In china, the plant is used traditionally as a diuretic, febrifuge (to treat fever) and as an anti-inflammatory agent. (HONG *et al.*, 2000); in Pakistan it is used as a fodder crop for animals and also eaten by humans as a vegetable. It is also used medicinally but as a laxative, to cure inflammations of the skin as well as leprosy (QAISER *et al.*, 1975). The people of Nepal eat the young leaves as a vegetable, use a paste from the plant to treat burns and the fluid from the roots is used to treat indigestion (MANANDHAR *et al.*, 2002).

Materials and methods

Collection and preparation of dried plant material: the plant raw material was collected from the wild and was authenticated by the Botany Department of Mbarara University of Science and Technology (MUST). It was collected during November 2008. The plant material was then dried under the shade and not the

sun so as to protect the thermo labile components if present from being chemically transformed. The plant material was then reduced in size by crushing it into smaller pieces using the hand. After the plant material had been dried, it was kept in a proper container until the time of the extraction (Fig. 1).

Collection and preparation of fresh plant material: the plant material was collected from the wild during the morning of the day of extraction. It was then authenticated by the botany department of MUST. Size reduction was performed by chopping the plant material into smaller pieces using a chopping blade. The plant material was then placed in a suitable container, ready for use that very afternoon for the extraction process.

Ethanol extraction (absolute alcohol): 100g each of the dried plant material and fresh plant material were weighed using a weighing balance. The plant material was then chopped into smaller pieces using a pair of scissors and placed separately into round bottomed flasks. The flasks were then filled with absolute alcohol; 850ml for the dry plant material and 500ml for the fresh plant material. The dry plant material needed more alcohol because it had to first absorb it then have an additional volume for wetting and soaking. These were left to stand for a period of 1 week. Volumes of solvents and weights of raw material were recorded in Table 1.



Figure 1. Plant of *Commelina benghalensis*

Concentration of the extracts: after 1 week, the two alcoholic extracts were obtained by sieving to separate the neat extract from the residue. This residue was then rinsed three times with portions of 50ml ethanol to ensure total extraction was done. The neat extracts were then distilled separately to movil residues and dried to dryness in a stove at a temperature of 50°C to obtain a dried extracts.

The percentage of extractives was calculated. The results were then recorded in Table 2.

Aqueous extraction (distilled water)

Maceration: 58g of the dried plant material and 100g of the fresh plant material were weighed using a weighing balance. The plant material was then chopped into smaller pieces using a pair of scissors and placed separately into round bottomed flasks. The flasks were then filled with distilled water; 300 ml for the dry plant material and 200 mls for the fresh plant material. Again, the dry plant material needed more solvent (water) because it had to first absorb it then have an additional volume for wetting and soaking. These were left to stand for 1 day. Volumes of solvents and weights of raw material were recorded in Table 3.

Concentration of the extract: after 1 day, the two aqueous extracts were obtained by sieving to separate the neat extract from the residue. This residue was then rinsed three times with portions of 50ml distilled water to ensure total extraction was done. The neat extracts were then dried to dryness in a stove at a temperature of 50°C to obtain dried extracts. The weight of the extract alone was determined and the percentage of extractives was calculated. The results were then recorded in Table 4.

Phytochemical screen: the four dried extracts were re-dissolved in 50ml of their respective solvents to obtain stock solutions for the phytochemical screen and antimicrobial assays. The strengths of the solutions were then calculated and recorded in tabular form (Table 5). In the phytochemical screen, 14 tests were performed on the ethanol extracts while 6 tests were performed on the aqueous extracts. For the extracts from the dried plant material, 2ml of the ethanol extract were placed in 14 test tubes and tested for the presence or absence of different chemical constituents. Similarly, 2ml of the aqueous extract were placed into 6 test tubes and tested for the presence or absence of different chemical constituents. The same procedure was followed for the extracts from the fresh plant material.

Reactions to be done with ethanol extract

Ethanol extract is divided into fractions:

2 mL to test Fehling Reag. For reducing agents determination.

2 mL for foaming test (saponins).

2 mL for ninhydrine test (amino acids)

2 mL to test phenols and tannins with ferric chloride solution.

2 mL to test quinones with Borntrager assay.

6 mL divided into 3 portions to test alkaloids with Dragendorff, Mayer and Wagner reagents.

2 mL for resin assay.

2 mL to determine lactone ring with Baljet reagent.

2 mL to determine triterpenoids and steroids with Liebermann-Burchard test.

2 mL to test flavonoids through out Shinoda assay.

Reactions to be done with aqueous extract

6 mL divided into 3 portions to test alkaloids with Dragendorff, Mayer and Wagner reagents.

2 mL to test phenols and tannins with ferric chloride solution.

2 mL to test flavonoids through out Shinoda assay.

2 mL to test Fheling Reag. For reducing agents determination.

2 mL for foaming test (saponins).

2 mL for mucilage assay.

1-2 drops for bitter and astringent principles assay.

The results obtained were then recorded in Table 6.

Antimicrobial assay on the plant extracts

Test microorganisms were obtained from the Department of Microbiology of Mbarara University of Science and Technology and were: *Staphylococcus aureus* (ATCC25923), *Candida albicans* (was an isolate from a patient) and *Escherichia coli* (ATCC 25922). Control antibiotics (Gentamycin for the bacteria and Nystatin suspension for the fungi) were used.

Preparation of stock solutions

The stock solutions of crude extracts from ethanol and water were made by dissolving 0.5g of the dry extracts in 1ml of a suitable solvent. From the stock solutions serial dilutions were made to obtain the test solutions of concentration 1/2, 1/4., 1/8, 1/16 and 1/32, 1/64, 1/128.

Procedure for antimicrobial tests

The volumes of extracts that remained after the phytochemical screening were used for the antimicrobial assay. The stock solutions of crude extracts from

ethanol and water were made by dissolving 1ml of the dry extracts in 1ml of a suitable solvent. From the stock solutions serial dilutions were made to obtain the test solutions of concentration 1/2, 1/4., 1/8, 1/16 and 1/32, 1/64, 1/128. The antimicrobial assay was performed by the agar well diffusion method. The agar was melted, poured on to the Petri dish and left to solidify. Using sterile swabs, the organisms were smeared on the solidified agar by streaking.

Candida albicans was incubated on Saboroud Dextrose Agar (SDA), *Staphylococcus aureus* and *Escherichia coli* were grown on Mueller- Hinton agar (MHA) which had previously been streaked with microorganisms at a density adjusted to 0.5 McFarland standards (108 colony-forming units [CFU]/ml).

A well was prepared in the plates with the help of cork-borer (0.03cm). 100µl of different concentrations of the test compound was introduced into the wells. The plates were incubated for 24 hours at 37°C. *Candida albicans* was incubated for seventy two hours at 27°C.

For each microbial species, negative control was maintained where 100µl of ethanol alone without the drug was used for ethanol extract. Also, conventional drugs were used for positive controls. In the central hole of the different Petri dishes, the control was put for each organism, Gentamycin for *Escherichia coli* and *Staphylococcus aureus* and Nystatin for *Candida albicans*.

The results were recorded by measuring the diameter of the zones of growth inhibition surrounding the wells (cylinders). The net effect of the drug extracts was obtained by subtracting the diameter of the zone of inhibition due ethanol alone from the diameter of the zone of inhibition due to the drug extract plus ethanol. However for the aqueous extracts, there was no need for subtracting the zones of inhibition due to ethanol. This procedure was repeated for all the 4 plant extracts and the results obtained were recorded.

Results

Ethanol extraction (absolute alcohol)

Table 1. Showing maceration of the ethanol extracts

	Dry plant material	Fresh plant material
Weight of plant material (g)	100	100
Volume of ethanol(mls)	850	500

Table 2. Showing concentration of the ethanol extracts

	Dry plant material	Fresh plant material
Net weight of the dried extract (g)	4.35	1.20
Percentage of extractives (%)	4.35	1.20

Aqueous extraction (distilled water)**Table 3.** Showing maceration of the aqueous extracts

	Dry plant material	Fresh plant material
Weight of plant material (g)	58	100
Volume of water (mls)	300	200

Table 4. Showing concentration of the aqueous extracts

	Dry plant material	Fresh plant material
Net weight of the dried extract (g)	5.53	1.24
Percentage of extractives (%)	5.53	1.24

Phytochemical screen

Table 5. Calculation of the strengths in % w/v of the four extracts after re-dissolving in 50mls of the respective solvents

Extract	Dry ethanol	Fresh ethanol	Dry aqueous	Fresh aqueous
Calculation	$(50/100) \times 4.35$	$(50/100) \times 1.20$	$(50/100) \times 5.53$	$(50/100) \times 1.24$
Strength (%w/v)	2.175	0.600	2.765	0.620

Table 6. Showing phytochemical screening for chemical constituents in the dry and fresh plant extracts

Assay / test/reagent	Chemical groups	Dry plant extract		Fresh plant extract	
		ethanol	aqueous	ethanol	aqueous
Sudan	Oils and fats	++	-	++	-
Dragendorff's	Alkaloids	++	-	++	-
Mayer's	Alkaloids	-	-	-	-
Wagner's	Alkaloids	-	-	-	-
Baljet	Lactones and coumarins	-	-	++	-
Lieberman-Burchard	Triterpenoids and steroids	+	-	++	-
Resin	Resins	+++	-	+	-
Fehling's	Reducing agents	++	++	+	++
Iron (III) chloride	Phenols and tannins	+	++	++	++

Ninhydrine	Amino acids	+	++	++	++
Borntgrager's	Quinones	+++	-	++	-
Shinoda	Flavonoids	++	-	+	-
Taste	Astringents	+	+	+	+
Foam	saponins	+	+++	++	+++

Key: (+) means the result is positive; (++) very positive; (+++) extremely positive ;(-) absent.

Antimicrobial assay in Tables 7- 10.

Table 7. Showing "in vitro" antimicrobial activity of the ethanol extract (2.175% w/v) from dried plant material

Micro organism	Diameter of zone of growth inhibition of controls and serial dilutions (mm)									
	Gentamycin or Nystatin	Neat extract	Absolute alcohol	1/2	1/4	1/8	1/16	1/32	1/64	1/128
<i>Escherichia coli</i> ATCC 25922	40	16	4	12	12	10	11	8	6	4
<i>Staphylococcus aureus</i> ATCC 25923	48	18	6	17	12	10	10	10	9	8
<i>Candida albicans</i>	22	12	7	12	12	12	11	11	9	9

Table 8. Showing "in vitro" antimicrobial activity of the ethanol extract (0.6% w/v) from fresh plant material

Micro organism	Diameter of zone of growth inhibition of controls and serial dilutions (mm)									
	Gentamycin or Nystatin	Neat extract	Absolute alcohol	1/2	1/4	1/8	1/16	1/32	1/64	1/128
<i>Escherichia coli</i> ATCC 25922	40	17	5	17	18	14	10	10	9	8
<i>Staphylococcus aureus</i> ATCC 25923	46	11	3	12	11	11	11	10	10	7
<i>Candida albicans</i>	20	19	6	19	19	18	18	17	15	10

Table 9. Showing "in vitro" antimicrobial activity of the aqueous extract (2.765% w/v) from dry plant material

Micro organism	Diameter of zone of growth inhibition of controls and serial dilutions (mm)									
	Gentamycin or Nystatin	Neat extract	1/2	1/4	1/8	1/16	1/32	1/64	1/128	
<i>Escherichia coli</i> ATCC 25922	42	10	0	0	0	0	0	0	0	
<i>Staphylococcus aureus</i> ATCC 25923	44	12	0	0	0	0	0	0	0	
<i>Candida albicans</i>	17	0	0	0	0	0	0	0	0	

Table 10. Showing “in vitro” antimicrobial activity of the aqueous extract (0.62% w/v) from fresh plant material

Micro organism	Diameter of zone of growth inhibition of controls and serial dilutions (mm)								
	Gentamycin or Nystatin	Neat extract	1/2	1/4	1/8	1/16	1/32	1/64	1/128
<i>Escherichia coli</i> ATCC 25922	40	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i> ATCC 25923	52	13	0	0	0	0	0	0	0
<i>Candida albicans</i>	20	0	0	0	0	0	0	0	0

The serial dilutions of absolute alcohol gave no zones of growth inhibition for all the three micro organisms. Hence the zones of inhibition observed for the serial dilutions are due to the plant extract alone.

Discussion

In African traditional medicine practice, southwestern Uganda inclusive, the traditional healers usually use the extracts from the freshly collected plant material to prepare their concoctions. However, the results obtained above indicate that the yield from extracts of the dried plant material; 4.35% for ethanol extract and 5.53% for aqueous extract is far greater than that from the fresh plant material; 1.20% for ethanol extract and 1.24% for aqueous extract simply because the quantity of water already present in the fresh plant material reduces the true weight of the material as compared to that of dried plant material. Only about 1/5 of the true weight of fresh material is used for the extraction. Hence using dried plant raw materials is more efficient for the extraction with ethanol because they contain trace amounts of water in their cells or tissues. It is also important to note that the yield from the aqueous extracts; 5.53% for dried plant material and 1.24% for the fresh plant material is far greater than that from the ethanol extracts; 4.53% for dried plant material and 1.20% for fresh plant material, thus indicating that perhaps water is a better solvent if one is aiming at obtaining large quantities of extractives. However, a high yield of extractives does not mean a higher biological activity.

From Table 6, it will be noted that the ethanol extracts give more positive tests for different chemical constituents; 11 for the ethanol extract of dried plant material and 12 for the ethanol extract of fresh plant material as opposed to 5 for each of the aqueous extracts of dried plant material and fresh plant material. The traditional healers make use of water primarily as a solvent but this study has shown that the ethanol extract of *Commelina benghalensis* contains more of the pharmacologically active secondary metabolites. This is because most of these secondary metabolites being organic in nature are soluble in ethanol

which is an organic (moderately polar) solvent and not in water, a more polar solvent. Hence successful extraction of phytochemical compounds from plant material is largely dependant on the type of the solvent used in the extraction procedure.

The results of phytochemical screening from Table 6 show that *Commelina benghalensis* contains 10 main secondary metabolites of pharmacological importance namely alkaloids, lactones and coumarins, triterpenes and steroids, resins, reducing agents, phenols and tannins, amino acids, quinones, flavonoids and saponins. No other group of natural products has contributed to medicines and pharmaceutical preparations than alkaloids. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup family, are commonly found to have antimicrobial properties (OMULOKOLI *et al.*, 1997). As a group, alkaloids display an exceptionally wide array of biological activities and a wide distribution. Flavonoids have important dietary significance because; being phenolic compounds they are strongly antioxidant and this probably explains why in Pakistan and Nepal the young leaves of *Commelina benghalensis* have been eaten as vegetables. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that flavonoids have been found “in vitro” to be effective antimicrobial substances against a wide array of microorganisms (DIXON *et al.*, 1983). Coumarin was found “in vitro” to inhibit *Candida albicans* (THORNES *et al.*, 1997) and this probably explains why the ethanol extract from fresh plant material of *Commelina benghalensis* that contained coumarins was more active against *Candida albicans* than the other plant extracts. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins (HASLAM *et al.*, 1996).

Terpenes or terpenoids are active against bacteria, fungi, viruses, and protozoa and it has been reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (CHAURASIA *et al.*, 1977).

Tables 7 and 8 indicate that serial dilutions of the ethanol extracts give a progressive reduction in the zones of inhibition and this is due to a reduction in the concentration of the chemical constituents.

From Table 7, comparing the dilution of 1/32 with the neat extract, *Escherichia coli* gives activity of 50%, *Staphylococcus aureus* 56% and *Candida albicans* 91%. Comparing the neat extract with the standard drug, *Escherichia coli* gives activity of 40%, *Staphylococcus aureus* 37.5% and *Candida albicans* 54.5%. This indicates that the activity of the extract against *Candida albicans* > *Escherichia coli* > *Staphylococcus aureus*. Hence the ethanol extract from the

dried plant material of *Commelina benghalensis* is mostly active against *Candida albicans*.

From Table 8, comparing the most dilute solution (1/128) with the neat extract, *Escherichia coli* gives activity of 47%, *Staphylococcus aureus* 63.6% and *Candida albicans* 52.6%. Comparing the neat extract with the standard drug, *Escherichia coli* gives activity of 42%, *Staphylococcus aureus* 24% and *Candida albicans* 95%. This indicates that the activity of the extract against *Candida albicans* > *Escherichia coli* > *Staphylococcus aureus*. Hence the ethanol extract from the fresh plant material of *Commelina benghalensis* is mostly active against *Candida albicans*. The very high activity in comparison to the standard drug is probably because it is the only extract of the four that contains coumarins which have been found to inhibit *Candida albicans* "in vitro" (THORNES *et al.*, 1997).

Tables 9 and 10 indicate that serial dilutions of the aqueous extracts lose their activity completely. This is because the aqueous extracts have very few chemical constituents (only 5) as seen earlier and diluting them profoundly diminishes their pharmacological properties.

From Table 9, it will be noted that the activity of the aqueous extract from dried plant material of *Commelina benghalensis* is 24% for *Escherichia coli* and 27% for *Staphylococcus aureus*. Hence the activity of the extract against *Staphylococcus aureus* > *Escherichia coli*.

No activity was observed against *Candida albicans*. Hence the active compounds against *Candida albicans* were not extracted by water and activity against bacteria reduced by 40-50% and was only present in high concentrations of the extract.

Table 10 indicates that the activity of the aqueous extract from fresh plant material of *Commelina benghalensis* against *Staphylococcus aureus* is only 25% of the standard drug. No activity was observed against *Candida albicans* and *Escherichia coli*.

In general terms, results from the antimicrobial assay indicate that the ethanol extracts are superior in performance to the aqueous extracts. This has been attributed to the better solubility of active components in ethanol, an organic solvent. The ethanol extracts showed relatively comparable activities to Gentamycin and Nystatin which are locally available standard antimicrobial agents on the market. Ethanol extracts of the plant allow extraction of chemical components that are active against *Candida albicans* and less active against *Escherichia coli* and *Staphylococcus aureus*. These chemical compounds are not extracted with water but with ethanol. So the ethanol extract is the best if the

plant is going to be used for antimicrobial purposes. The traditional claims attributed to this herbal drug by the local people for the treatment of wounds, burns, conjunctivitis and ear infections are justified by the different degrees of antimicrobial activities exhibited by the aqueous extracts against *Escherichia coli* and *Staphylococcus aureus*.

These observations can be rationalized in terms of:

- ✓ The polarity of compounds being extracted by the solvent.
- ✓ The intrinsic bioavailability of extracted compounds.
- ✓ The ability of these compounds to dissolve or diffuse in the different media used in the assay.

It is also very important to note that in African traditional medicine practice, the traditional healers usually use the extracts from the freshly collected plant material to prepare their concoctions. However, the results obtained in this study indicate that the yield from extracts of the dried plant material; 4.35% for ethanol extract and 5.53% for aqueous extract is far greater than that from the fresh plant material; 1.20% for ethanol extract and 1.24% for aqueous extract simply because the quantity of water already present in the fresh plant material reduces the true weight of the material as compared to that of dried plant material. Only about 1/5 of the true weight of fresh material is used for the extraction. Hence using the shade dried plant material yields more extractives than using freshly collected plant material.

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