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**ASSESSMENT OF THE PROXIMATE, PHYTOCHEMICAL AND ANTI-FUNGAL POTENCY OF CRUDE LEAF EXTRACT OF *Asystasia gangetica***

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**ABSTRACT**

Studies on the anti-fungal potency of *Asystasia gangetica* on some fungi isolated from bread samples were conducted. Fresh leaf samples of *A. gangetica* were harvested from a botanical garden in Port Harcourt, washed and rinsed in distilled water. The leaf samples were sundried for 8 hours and crushed into powder and bottled in an air tight container for further studies. Stored cultures of *Rhizopus stolonifer*; *Aspergillus niger*, *Aspergillus flavus* and *Sclerotium rolfsii* isolated from bread were used as the test fungi. Four quantities (2 g, 3 g, 4 g, and 5 g) of the leaf powder were weighed out with the sensitive balance and used for the study. Each of the quantity was mixed with 10 ml of distilled water to produce various solutions and concentrations (20%, 30%, 40%, and 50% respectively) of the leaf extract. Blotter papers dampened in them respectively and placed in Petri dishes and labeled accordingly. One gram (1 g) each of the mycelia of the various fungi was placed on the blotter papers in Petri dishes in triplicate and incubated at room temperature of 25° C ± 3°C for 7 days. It was observed that the crude extract of *A. gangetica* showed 100% inhibition on *A. niger*, and *A. flavus* irrespective of the various concentrations. The rate of inhibition for *S. rolfsii* was very low at 20 and 30% concentrations. Similarly, the rate of inhibition of *R. stolonifer* was less compared to the rate of inhibition at 40% and 50%. However, at higher concentrations of 40% and 50%, the inhibition rate was higher (100%) as evidenced by the inability of the fungus to grow at these concentrations. Analysis of the photochemical composition of the leaves showed that the plant is rich in several food nutrients such as moisture, ash carbohydrate, fibre nutrients and vitamin C. Mineral constituents of the plant include calcium, iron, potassium, sodium and phosphorus other phytochemicals include Tannins, Saponins, oxalates and cynogenic glycoside which performs numerous functions against microbial agents.

**Keywords:** *Asystasia gangetica*, leaf extract, bread, anti-fungi, potency, photochemical.

## INTRODUCTION

*Asystasia gangetica* popularly known as Chinese violet, creeping foxglove, Ganges prime rose, autumn belle and *Asystasia* is an herbaceous weed that grows along roadsides, rubbish dumps, vacant residential lands, waste lands and in open areas along jungles (Gann *et al.*, 2001, Pooley, 1998). It is believed to have been introduced to Malaysia from Africa and Asia over ninety years ago and has become a serious weed in most of the plantations found in Malaysia. This weed is quite unknown in several areas and as such is quite unpopular. It grows prolifically and tolerates shades and produces numerous seeds. Once established spreads rapidly and aggressively and could become very difficult to eradicate (Rauch, 1997). It has the capability of sprawling on the ground and along fence for support. It is a successful colonizer that has spread across different geographical locations because of its easy establishment, fast growth rate and easy flowering (Chuku *et al.*, 2018).

Some of the uses of this potent herb are as follow: Leaves and young shoots are cooked, leaves and flowers

are eaten as pot herbs (Grubben *et al.*, 2004). The leaves are also consumed as vegetables mixed with beans, groundnuts and other legumes the plant is also used traditionally as medicine in many parts of the world. The plant sap is used as a vermifuge and is applied externally to swellings and rheumatic joints. The sap is put on nose to stop nose bleeding. A leaf decoction is used in the treatment of fever-aches, epilepsy, stomach pains, heart pains and urethral discharge. The pulped leaf is used as suppository for piles, treatment of asthma (Akah *et al.*, 2003), and eases child birth by boiling and mixing it with pepper then given to the pregnant mother through syringe in later pregnancy as enema to ease child birth. The infusion is also taken orally for the same purpose (Ida and Onyibe, 2007). In Congo the leaf sap placed on the stomach of women in child birth to facilitate labour.

Generally, microorganism are adjudged the most notorious culprits, amongst other factors, responsible for post harvest diseases of crops (Ray *et al.*, 2000) and fungi in particular, have been found to be one of the principal causes of post harvest losses

in many zones of the world, ranking alongside insects and pest (Greer, 1990). Some fungi have been implicated to be highly toxic, carcinogenic and able to also suppress one's immune system, cause growth retardation, liver disease and death in birth humans and domestic animals (Bankole *et al.*, 2005). Seeking ways to effectively control post harvest spoilage of agricultural produce is therefore undeniably essential.

The use of synthetic chemicals in the preservation of post harvest agricultural produce in storage has proven over the years to be very effective in controlling pathogenic fungi (Manczinger *et al.*, 2002). Notwithstanding the pedigree of effectiveness, their use is increasingly becoming undesirable because they are themselves carcinogenic, teratogenic, highly toxics and able to induce chemical poisoning, as well as fungal resistance, (Adegoke *et al.*, 2002). As a result search for post harvest control strategies has recently been directed toward the use and implementation of natural preservatives that may have a positive effect on human health (Burt, 2004; WHO 2002).

Amongst natural preservatives, the use of natural essential oils obtained from plants have been promising (Ebimieowei and Emiri, 2016). They have been shown to reduce microbial and chemical spoilage among agricultural produce (Pessoa, 2002) and more importantly, they are eco friendly, with no proven detrimental effect on human health and the environment even at high concentration (Nychas, 1995; Smid and Gorris, 1999). Amongst such proven botanicals is *Asystasia gangetica*. There is dearth of information on the use of *A.gangetica* extract as an anti-fungal plant, which formed the basis for this study.

In this work, the proximate composition and phytochemical properties of *Asystasia gangetica* was studied along side and the anti-fungal activity of the test plant extract against spoilage fungi isolated from food substance. The findings from this work would provide simple ways to reduce the health hazards associated with the consumption of post- harvest food crops.

## MATERIALS AND METHODS

### Sample Collection

Fresh leaf samples of *A. gangetica* were harvested from a garden in the Rivers State University, Port Harcourt, washed and rinsed in distilled water. The leaf samples were sun-dried for 8 hours and crushed into powder and bottled in an air tight container for further studies. Stored cultures of *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Sclerotium rolfsii* isolated from bread were used as the test fungi

### Phytochemical Analysis

The plants were allowed to establish fully before the leaves were collected for Phytochemical analysis. At the sixth month after planting, the leaves were harvested and taken to the Food Science and Technology Laboratory for phytochemical analysis using the AOAC (2006) methods of analysis.

### Preparation of Medium

Sterilization of conical flask, slides, Petri-dishes and all the equipment needed for the experiment was carried out in the laboratory using the standard methods (Agrios, 2005). The mycological medium used was Sabouraud Dextrose Agar prepared

in a conical flask using the standard method (Agrios, 2005).

### Isolation of Fungi

Fungi were isolated from small samples of bread using the Scottie tissue method. The isolated fungi were inoculated on to Sabouraud Dextrose Agar in Petri-dishes onto which ampicillin was added to inhibit the growth of bacteria. The inoculation was done in triplicate. The Sabouraud Dextrose Agar was prepared according to manufacturers specification. The inoculated plates were incubated for 5 days at ambient temperature of  $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$ . (Baudoni, 1988, Chuku, 2009, Samson *et al*, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates were obtained after series of sub-culturing and the extent of fungal growth was determined with a meter rule and converted to percentages the (Onuegbu, 2002).

### Identification of Fungal Organisms

Microscopic examination of the fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread

spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colony morphology, mycelia structure and other associated structures using the keys of (Samson *et al*, 1981 and Olds, 1983).

### **Inhibitory Effect of Leaf Extracts of *Asystasia gangetica* on Fungal Isolates**

The inhibitory effect of aqueous leaf extracts of *Asystasia gangetica* on growth of four test fungi. (*R. stolonifer*, *A. Niger*, *A. flavus* and *S. rolfssii* previously isolated from bread was studied. Four quantities (2 g, 3 g, 4 g and 5 g) of the sensitive balance. Each of the quantity was mixed with 10ml of distilled water to produce various solutions and concentrations (20%, 30%, 40% and 50% respectively) of the leaf extract. Blotter papers dampened in them respectively and placed in Petri dishes and labelled accordingly. One gram (1 g) each of the mycelia of the various fungi was placed on the blotter papers in Petri dishes in triplicate and incubated at room temperature of  $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$  for 7 days. This procedure was

repeated for distilled water. The inhibitory effect of the test plant extracts and control treatments were ascertained by a clear zone of inhibition of fungal growth on the Petri dish after 5 days of incubation. The distilled water was to serve as control.

## **RESULTS**

### **Proximate Composition of the Leaves of *A. gangetia***

Results of the proximate composition of *A. gangetia* are presented in Table 1. The leaf samples of *A. gangetia* contain high amount of moisture and vitamin C. Other nutrient composition such as ash, lipid, carbohydrate, fibre and protein were also present in appreciable quantity.

Mineral composition of *A. gangetica* is represented in Table 2. The fresh leaf samples of *A. gangetica* contain essential elements needed for healthy growth and development in human. Such major mineral components include calcium, phosphorus, sodium, iron and potassium.

Anti-nutritional components of *A. gangetica*. Other phytochemicals analysed include the anti-nutritional constituents of *A. gangetica*. The

plant is found to contain tannins, saponins, oxalates and cynogenic glycosides.

### **Shelf life Studies of *A. gangetica***

Results of the self-life studies of *A. gangetica* is presented in Table 4 – 6. Dried powdered leaf samples of *A. gangetica* recorded low moisture value against the fresh sample. The values of ash, lipid carbohydrate, fibre and protein were also higher in dried leaf sample than that of fresh samples.

The mineral composition of dried leaf samples of *A. gangetica* increased more than was recorded for the fresh leaf samples. However, it was observed that potassium concentration in dried leaf samples was very high ( $25.5 \pm 0.01$ ) compared to what was recorded for fresh samples ( $2.83 \pm 0.01$ ). All other mineral components of the dried leaf samples of *A. gangetica* slightly increased.

Anti-nutrient properties of both the fresh and dried leaf sample of *A. gangetica* did not vary much except for tannins which increased from ( $0.09 \pm 0.04$ ) in fresh leaf samples to ( $0.88 \pm 0.02$ ) in dried leaf. Comparative effects of different Concentra-

tions of Plant (*A. gangetica*) extracts are presented in Table 7.

### **DISCUSSION**

Almost every plant that grows around our environment is useful either as a vegetable or a medicinal herb (Onuegbu, 2002). The only constraint we have is our limited knowledge of the uses of plants found in our vicinity. The early man consumed most of the plants and fruits found in the wild for so many decades before the conscious and deliberate effort by man to cultivate these plants. However, a lot of literature abound on the nutritional qualities of most tropical vegetables, fruits and nuts (Achinewu, 1996, Chuku and Ugorji, 2012).

The proximate composition of *A. gangetica* revealed the presence of moisture and other essential nutrient elements an indication that it is a potent vegetable that could be consumed to obtain a balanced diet. The high moisture, protein and vitamin c contents of this plants is an indication of the potency of this plant as a diet for weight loss and an antioxidant that could boost the human immune system thereby preventing the accumulation of certain unwanted food

materials in the body that can lead to serious health challenge. The results obtained from this study further buttressed the fact that *A. gangetica* could be consumed as a vegetable in a similar way *Vernonia amygdalina* is consumed (Grubben and Denton, 2004).

The plant also contains vital mineral elements such as calcium and potassium for strong building of the body frame work (Idu and Onyibe, 2007). Sodium and phosphorus help in balancing electrolytes in the body and regulates the body sugar while Iron is the hemoglobin booster (Kin and Onyibe, 2007). The consumption of this plant promotes healthy living which may be the reason why the local people cherish it Apart from the nutrient constituents of this plant, it also contains some anti-nutrient elements such as tannins, saponnins, oxalates and cynogenic glycosides. These anti nutrient elements help to protect the plants from herbivores especially the tannin and saponnins which deter the consumption of this plants by animals. For the shelf life preservation, drying of fruits, vegetables and herbs is a very easy process

and can be done without any special equipment or expertise.

The essence of drying is to reduce the moisture contents of these materials to their safe level bearing in mind that high relative humidity and moisture encourage the deterioration of most agricultural produce leading to microbial infection (Chuku *et al.*, 2004). Shelf life study also revealed that most of the nutrient as shown in the proximate composition became more concentrated and increased in values. The protein content, ash, lipid, carbohydrate and fibre increased an indication of quality stability. The explanation here is that drying the leaf of *A. gangetica* gave rise to increased nutrient quality accompanied by reduction in moisture content. Other Phyto-chemicals such as the mineral components of *A. gangetiea* also increased considerably in dried leaf samples than the records from the fresh leaf samples.

Result on the potential use of *A. gangetica* as an organic fungicide showed that aqueous crude extracts of *A. gangetica* at various concentration (20%, 30%, 40% and 50%) separately inhibited the growth of all

test fungi in comparison to the control (Water) treatment. *A. gangetica* is found throughout the tropics and subtropics, including Nigeria (Chuku *et al.*, 2018). However, this work is the first report where its antifungal potency was assessed.

Antimicrobial properties of *A. gangetica* are attributed to phytochemicals and essential oil components which they possess. Phytochemicals and essential oil are secondary metabolites that are non-nutritive in themselves but have been found to confer on plants that possess them the ability to resist microbial infections, and sometimes environmental stress. (Prably *et al.*, 2009; Ladipo *et al.*, 2010, Matasyoh *et al.*, 2007).

The fungal inhibitory effect of extracts of *A. gangetica* in this study was found to be dose dependent. While the overall mean diameter of zone of inhibition across all four test fungi was 86.87%, the plant extract at 50% concentration had a greater inhibitory effect on the fungi (100%) than at 20% and 30% concentration (72.5% and 75.0%) respectively (Table 7). The pattern of correspondingly greater rate of inhibition with

respect to increase in concentration of the plant extract was similar to results reported by several other workers (Ijato, 2011). These workers separately reported that an increase in the concentration of an antimicrobial plant extract achieved a better result in the control of pathogenic fungi. Tagne *et al.*, (2000) also reported that plant extract at 10ppm exhibited a considerable degree of antifungal activity but test fungi were completely inhibited when concentration of extract was increased to 800ppm.

Result from this present work also showed that the degree of antifungal activity of extract of *A. gangetica* was different from the different test fungi. Antifungal activity of the extracts was most active against *A. species* and least in *Sclerotium rolfsii* (Table 7).

The differential antifungal effects of *A. gangetica* on *Aspergillus* spp. and *S. rolfsii* could not easily be fathomed. Different workers at different time have shown that *Aspergillus species* respond differently to antifungal agents. An antifungal substance could be active against

*Aspergillus species* but not *Sclerotium* spp. and vice versa.

The inhibitory effect of the test plant extract against *Aspergillus species* confirms earlier work done by Etebu and Emiri (2016) which revealed that antifungal activity of botanical extracts (*Ocimum gratissimum*) was most active against *Aspergillus species*. The low inhibitory effect of plant extract on *S. rolfsii* Suggest that it is resistant to antifungal agent. This confirms the assertion of earlier workers who demonstrated the efficacy of some synthetic fungicide on *S. rolfsii*, it was observed that all fungicides used during the study showed a gradual decline in the growth of *S. rolfsii* with increase in concentration in the medium. (Ohazuruike 1996). The result from this present work suggest that *A. gangetica* may be more effective if used in the control of post harvest diseases occasioned by *R. stolonifer* and *Aspergillus species* than by those caused by *sclerotium species*.

The increased level of potassium of dried leaf of *A. gangetica* suggests that the increase was on account of drying. Apparently, the potassium

inherent in dried leaf of *A. gangetica* was responsible for the inhibition of test fungi. Again, increased doses of the plant extract inhibited entirely, the test fungi. This confirms the assertion of Ortega-Aguilar (2011), who reported the inhibition of fungal growth and *sclerotia* of *Sclerotium ceptivorum* by potassium bicarbonate (KHCO). He observed that an increased dose of KHCO at 50 mm the growth of the fungal culture was entirely inhibited. Reduction of soil fungal population in potassium rich soil has been reported as well (Emiri *et al.*, 2012). The report agrees with the findings of this study.

## CONCLUSION

The current status of research suggests that there are indeed alternatives to replace the synthetic fungicides for management of notorious seed borne fungi. It is possible that adopting botanical extracts approach as evidenced in *A. gangetica*, eco-friendly and economically viable alternative for crop production system can be developed.

**Table 1:** Proximate Composition of fresh leaf samples of *A. gangetica*

<b>Nutrient Elements</b>	<b>%</b>
Moisture	70.9 ± 0.04
Ash	2.5 ± 0.01
Lipid	2.63 ± 0.01
CHO	5.83 ± 0.06
Fibre	5.7 ± 0.03
Protein	12.5 ± 0.02
Vitamin C	35.7 ± 0.01

P ≤ 0.05 , ,  $\pm$  = Standard Error

**Table 2:** Mineral Composition of fresh leaf samples of *A. gangetica*

<b>Mineral Composition</b>	<b>mg/ 100g</b>
Calcium	0.72 ± 0.13
Phosphorus	0.66 ± 0.11
Sodium	1.77 ± 0.23
Iron	0.23 ± 0.21
Potassium	2.83 ± 0.01

P ≤ 0.05 , ,  $\pm$  = Standard Error

**Table 3:** Anti-Nutritional Components of fresh leaf samples of *A. gangetica*

<b>Anti-Nutritional Components of <i>A. gangetica</i></b>	<b>mg /100g</b>
Tannins	0.09 ± 0.04
Saponins	1.96 ± 0.01
Oxalates	0.60 ± 0.33
Cynogenic glycosides	0.54 ± 0.11

P ≤ 0.05 , ,  $\pm$  = Standard Error

**Table 4:** Proximate Composition of Powdered dried leaf of *A. gangetica*

<b>Nutrient Elements</b>	<b>%</b>
Moisture	22.5 ± 0.02
Ash	4.38 ± 0.01
Lipid	3.65 ± 0.03
CHO	36.75 ± 0.05
Fibre	12.54 ± 0.03
Protein	20.17 ± 0.02
Vitamin C	30.6 ± 0.01

P ≤ 0.05 , , ± = Standard Error

**Table 5:** Mineral Composition of Dried Leaf Sample of *A. gangetica*

<b>Mineral Composition</b>	<b>mg/100g</b>
Calcium	0.95 ± 0.13
Phosphorus	0.67 ± 0.11
Sodium	1.80 ± 0.23
Iron	0.4 ± 0.21
Potassium	25.5 ± 0.01

P ≤ 0.05 , , ± = Standard Error

**Table 6:** Anti-Nutritional Composition of fresh dried leaf samples of *A. gangetica*

<b>Anti-Nutritional Components of <i>A. gangetica</i></b>	<b>mg/100g</b>
Tannins	0.88 ± 0.02
Saponins	2.0 ± 0.01
Oxalates	0.60 ± 0.03
Cynogenic glycosides	0.75 ± 0.01

P ≤ 0.05 , , ± = Standard Error

**Table 7:** Comparative Effects of different Concentrations of Plant (*A. gangetica*) Extracts on Fungal Isolates Zone of inhibition (%)

Treatment	<i>Rhizopus stolonifer</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>S. rolfsii</i>	Mean
Water	0.00 ± 0.00	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.0	0.00
Plant extract (20%)	80 ± 0.041	100 ± 0.02	100 ± 0.03	10.00 ± 0.04	72.5
Plant extract (30%)	90 ± 0.04	100 ± 0.01	100 ± 0.02	10.00 ± 0.06	75.0
Plant extract (40%)	100 ± 0.04	100 ± 0.03	100 ± 0.02	100.00 ± 0.10	100
Plant extract (5%)	100 ± 0.01	100 ± 0.04	100 ± 0.07	100 ± 0.12	100
<b>Mean</b>	<b>92.5</b>	<b>100</b>	<b>100</b>	<b>55</b>	<b>86.87</b>

$P \leq 0.05$  , ,  $\pm$  = Standard Error

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