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Phytochemical And Antimicrobial Activity Screening of *Indigofera Australis*

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ABSTARCT
The plant Indigofera australis was studied to determine the antibacterial activities and phytochemicals content in the crude extract of its leaves. The phytochemical components such as alkaloids, flavonoids, saponins, tannin, phenols, and hydrogen cyanide (HCN) in Indigofera australis leaves from Ohaozara Local Government Area of Ebonyi State, Nigeria were determined using standard methods. The antibacterial activities of the organic solvent extracts of the plant was determined by disc diffusion and broth dilution techniques against gram-positive bacteria strain (Staphylococcus aureus) and gram-negative bacteria strain (Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa).The results showed the concentration of the phytochemicals (in mg/100g) to be 0.93±0.05, 0.82±0.05, 0.32±0.05 0, 0.54±0.00, 0.72±0.01, and 0.00 ± 0.001 for alkaloids, flavonoids, saponins, tannin, phenols, and hydrogen cyanide (HCN) respectively. On the other hand, the results of the antimicrobial tests showed that the most susceptible organism to the organic extract among the test organisms was P. aeruginosa while the most resistant was E. coli. These results suggest that Indigofera australis is an ecofriendly bio poison and as such could be used in activities like fishing, if need be, rather than the common use of chemicals which normally turn out to pollute the environment.

Keywords: *Indigofera australis,* phytochemicals, antibacterial, ecofriendly, bio poison

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INTRODUCTION

Plants are invaluable sources of pharmaceutical products and Nigeria, in particular, has yielded an incredible array of plant and animal products that have drawn the attention of ethnopharmacologists from around the world. African medicinal plants have a long history of providing important sources of healing drugs to local populations (Brain and Turner, 2010). In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines. *Indigofera australis* is a small to medium sized shrub, often of upright and straggly habit.
The leaves are pinnate, 50-100mm long, with up to 21 egg-shaped to narrowly oblong leaflets which are slightly hairy. The flowers occur in elongated clusters from the leaf axils. They are pea-shaped, pink or purple in color (occasionally white) and about 12mm across. Pea flowers consist of

4 petals; the "standard", the "keel" and two "wings". Flowering occurs in late winter and spring (Metcalfe and Chalk, 1981).

Indigofera Australis is widespread in open forest and woodlands in all states of Australia from the south-east of Western Australia to north-east Queensland. *Indigofera* is genus of about 700 species of which about 30 are found in Australia. The most commonly encountered species is *I.australis*. The common name is Austral Indigo (Johansen, 2000). The species varies greatly in size, habit and color, which is symptomatic of its geographical spread. *Indigofera australis* Kingdom: Plantae, Order: Fabales, Family: Fabaceae, and Genus: *Indigofera*. Leaves are pinnate, openly spaced on the stems, around 10cm long and velvety smooth to the touch. Their appearance is clean and fresh at all seasons, unblemished by pests and diseases, and their beautiful blue-green colour is most apparent during colder months. Flower colour is unusual, ranging through soft purple hues, often pinkish and a change from other species flowering at the same time. The flowers are smooth, in short spires in the leaf axils, freely produced and showy, outlining the curves of the stems (Lewis and Elvin-Lewes, 2007). The attractive flowers and the plants adaptability to grow in different situations make it suitable as an ornamental plant in Australia. The Australian aborigines crushed the leaves and added these to water to kill or stun fish and eels. The leaves and stems produce yellow-fawn dye with alum as mordant (Brain and Turner, 2010). Of the various *Indigofera* species, *Indigofera tinctoria* and *Indigofera suffruticosum* are especially used to produce the dye indigo (Lewis and Elvin-Lewes, 2007). Several species of this group are used in anticancer therapy (Evans, 2003). The herbs are generally regarded as an analgesic with anti- inflammatory activity. *Indigofera articulate* is used for toothache and swellings. *Indigofera aspalathoides* have also been used as anti – inflammatories *(Indian Pharmacopoeia,* 2006).

Phytochemicals are natural bioactive compounds which are present in plants. These natural compounds work with nutrients and dietary fibres to protect animals and man against diseases. (Alasbahi, *et al*.,1999). Medicinal plants constitute the main source of raw pharmaceuticals and healthcare products while also reported that extraction and characterization of several active phytocompounds from green plants have given birth to some high activity profile drugs (Alasbahi, *et al*., 1999). This work is therefore aimed at screening the levels of some phytochemicals in *Indigofera australis* leaves and investigating the antimicrobial activity of a crude extract of *Indigofera australis* leaves on some isolates of bacteria.

Fig 1: Flowered *Indigofera australis*

Fig 2: *Indigofera australis*

MATERIAL AND METHOD

Sample Collection

The *Indigofera autralis* leave used in this study were obtained from Isieke Oshiri in Onicha Local Government Area of Ebonyi State Nigeria and was at the Botany unit of the Department of Applied Biology of Ebonyi State University, Abakaliki Nigeria.

Sample of Preparation

The samples were washed under running water and at a temperature of 60^0C in an oven for two days before grinding. The ground samples were stored in air-tight containers until required for analysis.

Phytochemical Screening

The phytochemicals were screened using standard methods as described by Norris and Aksan, (2010). The samples were subjected to HCN test using the alkaline Picrate Colorimetric Method described by Metcalfe and Chalk, (1981).

Antimicrobial Activity Test

Preparation of Extracts

Fresh leaf of *Indigofera australis* was wash in a running water to remove dirts then air dried at room temperature for several days dried sample was grinded to fine powder and soaked in ethanol and water in a conical flask for 24 hours with interual stirring. The mixture were filtered using Whatman No. 1 filter paper Alasbahi, *et al*., (1999) into a clean beaker and the filterate was concentrated to dryness by evaporation using steam bath at 100^0C.

Test Organisms

The micro organisms used were staphylococcus *aureus*, Pseudomonas *aeruginosa*, Klebselia and E. *coli* (Escherichia coli). These were collect from BMD laboratory Ahiaeke Umuahia Abia State, Nigeria and were maintained on nutrient agar and the yeast on abouraud Dextrose agar slant after reactivation from the freeze dried materials.

Antimicrobial Assay

The disc diffusion method as reported by Alasbahi, *et al*., (1999) was adopted for the determination of antimicrobial activity of extract. Whatman No 1 filter paper was used with slight modification, while Microdilution Broth Assay was as described byEvans, (2003).

RESULT AND DISCUSSION

PHYTOCHEMICAL ANALYSIS OF *INDIGOFERA AUSTRALIS*

Analysis of the phytochemical contents of the leaf *Indigofera australis* showed that it contains Alkaloids, Flavonoids, Saponins, Tannins, Phenols and Hydrogen cyanide (HCN). The values of the phytochemicals are as shown in figure 1 below. Alkaloids had (0.93±0.05), Flavonoids (0.82±0.05), saponin (0.32±0.02), Tanins (0.54±0.00), phenol (0.72±0.01), while HCN was Seen to be very low in value (0.00 ± 0.0009) when compared with other parameters as shown in fig.

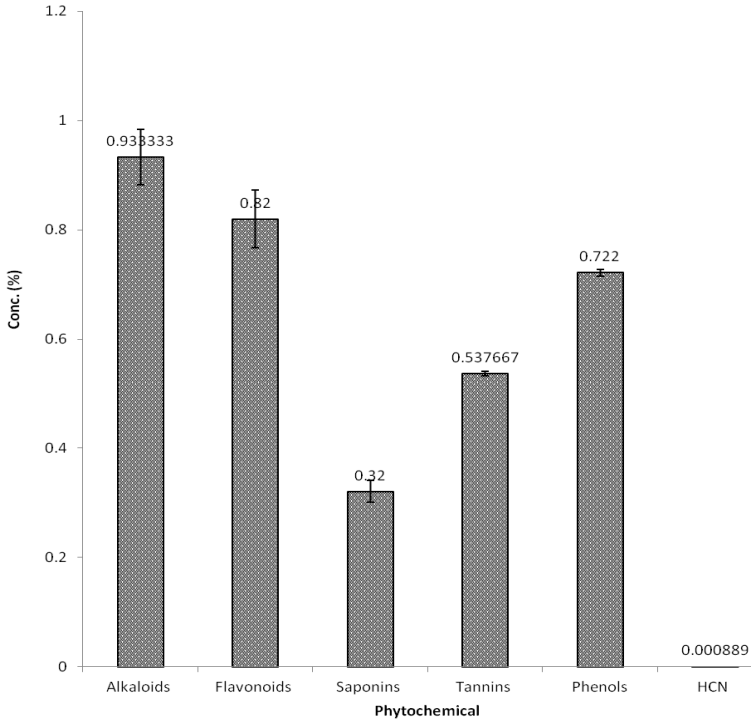

Figure 3: Phytochemical contents of *indigofera australis* from oshiri In Ebonyi State

Antimicrobial Analysis of *Indigofera australis*

The antimicrobial activities of the tested microorganism showed no inhibition with water extract in all isolates of microorganism as shown table 2. The microorganism was found growing when tested with water and strong inhibition was seen with ethanol extract the following microorganisms: *E. coli, Klebsiella and S. aureus;* indicate that the microorganism was inhibited from growth by the ethanol. Partial inhibition with *P. aeruginosa* it indicates that the ethanol has only little effect on the inhibition of *P. aeruginosa*

There was no sensitivity with ethanol and ampicillin in all the tested isolates, the microorganisms were resistant to ethanol and ampicillin, this is to say that plant extract and ampicillin cannot be used in the treatment of *Klebsiella, P. aeruginosa* and *S. aureus.*

But erothromycine is sensitivity to *E. coli* only but not sensitivity with other isolates (*Klebsiella* and *S. aureus*). From this result erothromycine can be used to treat *E. coli* but cannot be used to treat *Klebsiella* and *S. aureus* as showed in table 1 and 2.

Table 1: Antimicrobial Activities of water and ethanol extracts

Samples	E. Coli	Klebsellia	S. Aereus	P. Aeruginosa
Water Extract	No inhibition	No inhibition	No inhibition	No inhibition
Ethanol Extract	Strong inhibition	Strong inhibition	Strong inhibition	Strong inhibition

Table 2: Mean diameter of zone of inhibition (mm) of ethanol extract and standard antibiotics against the test bacteria.

Test bacteria	**Plant extract (2000µg/disc)**	**Ampicillin (10µg)**	**Erothromycine (15µg)**
E. coli	0	0	18
Klebsiella	0	0	12
S. aureus	0	0	16
P. aeruginosa	7	6	10

The quantitative phytochemical estimation present in *Indigofera Australis* studied showed that the leaves are very rich in saponins, Alkaloids, Tenins and flavonoids (figure 3). The presence and amount of tannins also confirms its astrigent property. This compound can also be effective in protecting the kidneys and it has also shown potential antibacterial and antiviral effects (Johansen, 2000). The saponin content makes the leaves an important source of detergents, surface active agents used in industrial applications and also possesses beneficial health effects (Brain and Turner, 2010). Most saponins, which readily dissolve in water, are poisonous to fish. This result therefore supports earlier report by Roldan and Varela, (1999) and affirms its role in fish poisoning. HCN content of *Indigofera australis* is also on the very high side as revealed from this study and shown

in Figure 1. Apart from the health benefits derived from HCN, they are also used in bleaching pulp for paper production because it is a building block of many dyes.

Results also revealed that Phenols is relatively high in the leaves of *Indigofera australis* (0.72±0.01%). This phytochemical has been known to be one of the most potent, rapidly acting poisons known (Johansen, 2000) and because the human body detoxifies it so rapidly; an adult can only withstand 0.05-0.06mg/g an hour without serious consequences. This suggests that the plant is not too safe for consumption even though it has some important bioactive compounds that may be beneficial to the body. Figure 1. also shows that the flavonoid content of *Indigofera Australis* relatively on the high side (0.82±0.05). This observation indicates that the plant has a high antioxidant effect. (Hegarty, 1990) earlier reported that flavonoids have antibacterial, anti-inflamatory, antiallergic, antimultagenic, antiviral, antineoplatic, anti-thrombotic and vasodilatory activities.

However, the low amount of alkaloid present (0.93±0.05mg/g) is also indicative of its harmless effect based on its content. Onyeka and Nwambekwe earlier reported that alkaloid content of some edible vegetables ranged between 12.8-29.6mg/g (Evans, 2003). Nisar and Tariq, (2011) had also pointed out that plants containing alkaloids do not feature strongly in herbal medicine because they are extremely toxic, yet, they have always been important in allopathic systems where the dose is strictly controlled and in homoepathy where the dose-rate is so low as to be harmless.

Phytochemical screenings are not only used to search for bioactive agents. Plants have provided agents which serves as starting products for the partial synthesis of some useful drugs. An example is the steroidal sapogenins produced by *Dioscorea* species (or Mexican yams) and also by the *Balanites* and *Trigonellai* species. The 'Solanum alkaloids' from *Solanum* species have been used in the partial synthesis of drugs (Johansen, 2000). Plant steroidal sapogenins are used as starting products in the synthesis of steroidal drugs such as corticosteroids, the sex hormones, and oral contraceptives.

The result showed that water encourages the activities of microorganism as it had no inhibitory effect on the growth of microorganism rather, encourage their growth. The microorganisms tested showed no inhibition with aqueous extracts, because water lack antimicrobial property. Ethanol has high antimicrobial activity as shown in table 2; the antimicrobial activity of ethanol extract is shown with strong inhibition (i.e. no growth of microbial in ethanol extract). However, the ethanol extract and ampicillin showed no sensitivity with *Klebsiella, P. aeruginosa, E. coli* and *S. aureus*. This means that the extract and ampicillin may not be suitable in the treatments against these microorganisms. Erothromycine can be used in the treatment of *E. coli* because the level of sensitivity of the antibiotic is high while the level of resistant of the *E. coli* is low compare with others. This work reveals that *Indigofera australis* possesses some important bioactive compounds which could be screened for several medicinal purposes.

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