Analysis of Phytochemical Content and Antibacterial Activity of *Tapinanthus dodoneifolius* Extracts

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Abstract: African mistletoe (*Tapinanthus dodoneifolius*) (DC), a plant parasite used ethnomedicinally for the treatment of several human and animal ailments including stomach ache, diarrhoea, dysentery, wound, cancer and hypertension was subjected to both phytochemical and antibacterial screening. The result of the phytochemical screening showed the occurrence of anthraquinones, saponins, carbohydrates, tannins, and alkaloids but absence of phlobatannins in the hemi-parasite. The *In vitro* assaying of the extracts using agar plate-hole and nutrient broth dilution techniques revealed a wide spectrum of antimicrobial activities against certain multiple drug resistant bacteria isolates with *Salmonella typhi* and *Staphylococcus aureus* being the most susceptible while *Bacillus subtilis* was the least. The inhibitory concentration (MIC) of the extracts ranged from 6.25 to 15.6mg/ml while the maximum bactericidal concentration (MBC) ranged from 25.0 to 62.5mg/ml. Interestingly, the antimicrobial activity of these extracts against the growth of *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Staphilococcus aureus* and *Pseudomonas aeruginosa*; the bacterial species known to be associated with either crown gall or gastrointestinal tract and wound infections gave credence to the ethno-medicinal usage of the plant. Since the antibacterial activities and the phytochemical constituents of *Tapinanthus dodoneifolius* could partly be dependent on the host plant species and since the locust beans tree upon which this plant grows has a wide variety of ethno-medicinal applications, the wide traditional applications of this parasitic plant could also be explained on this basis.

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1. Introduction

Before the availability of synthetic drugs, phyto-drugs or herbal drugs were the mainstay of treatment. Man has used plants to treat common infectious diseases. During recent years herbal medicine has become increasingly used in treating diseases. Due to demands from both the public and medical establishments, studies leading to the scientific explanation of plant therapeutic capabilities are allowing this practice to gain increasing credibility and acceptance. Therefore, some of the traditional medicines are still included as part of the habitual treatment of various maladies (Riossier., 2006).

Scientific interest in medicinal plants has burgeoned in recent times due to increased efficacies of their secondary metabolites and their derived products coupled with the rising concerns about the side effects of some modern medicines. The continuing emergence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacies of several antimicrobial agents currently in use. Therefore, the search for new drugs from novel sources such as plants continues to be necessary since they are the major sources of the basic primers of many synthetic drugs (Maureer-Grimes *et al*., 1996; Sofowara, 1982; Pascual *et al*., 2002).

Several factors, including lack of research and standardization in the third world have led to decline in phyto-medicine. Plants, however, still have bioactive secondary metabolites which if properly harnessed, could save these countries a great deal of the heavy healthcare budgetary allocations to imported synthetic drugs. This will also go a long way in reducing the emerging factors like drug-resistance by organisms and side-effects of synthetic drugs by the patients.

One plant of interest in the world is *Tapinanthus dodoneifolius.* This plant is a species of African mistletoe which is hemi-parasitic in nature. It grows on many trees and has ascribed medicinal uses (Ouedraogo *et al*., 2005; Aina *et al*., 2010; Inuwa *et al*., 2012). In Niger State, the leaves of this plant are extensively used locally for the treatment of several human and animal ailments that include stomach ache, diarrhoea, dysentery and wound. In Tangayika, the root decoction of this plant is used to treat hard abscesses while in Congo, its dried bark powder is used for the treatment of colds and sinusitis (Katsyal and Lamai, 2009). In Ayurvedic medicine, *Tapinanthus dodoneifolius* is used for the treatment of various diseases such as sciatica, chronic fever,

rheumatism, internal worm infections, asthma, inflammations, dyspepsia, dermatitis, bronchitis, cough, constipation, grayness of hair and baldness (Spencer, 2008).

This study investigated the phytochemical and antibacterial properties of the methanol, aqueous and chloroform leaf extracts of the medicinal plant against five bacterial isolates in order to expose new frontiers for the improvement on the current applications of this plant. Also, since it has been suggested that both the aqueous and alcoholic plant extracts used in allopathic medicine are potential sources of antiviral, antitumoural and antibacterial agents (Brain and Turner, 1975) and since the selection of crude plant extracts for screening programmes has the potential of being more successful at the initial steps than the screening of pure compounds isolated from natural products (Sofowora, 1993), this study looked into the effects of these crude extracts on the test organisms.

2. Materials and Methods

The plant samples (leaves) *Tapinanthus dodoneifolios* were collected during the rainy season in a farmland located in Bosso Area of Minna, Nigeria. They were found growing on the branches of the locust beans tree as parasites. They were collected in a substantial quantity and were air dried under room temperature, and pounded afterwards using pestle and mortar to increase surface area.

2.1 Extraction of Active Constituents

Aqueous Maceration Method

The cold maceration method was used to obtain the plant extracts. This was achieved by blending the air-dried powdered plant sample in 500cm^3 of distilled water. The blended mixture was allowed to stand for 24 hours. The suspension was then filtered and the filtrate concentrated over a water bath (Harborne, 1984). Using separating funnel, the concentrated extract was exhaustively extracted with chloroform to obtain the chloroform extract. Another portion of the fresh sample was cold extracted with 500cm^3 of methanol to obtain the methanolic extract. The three extracts were evaporated to dryness on a water bath,cooled and transferred into screw caped plastic containers, labeled and kept for further use.

2.2 Phytochemical Screening of Plant Samples

The aqueous, chloroform and methanolic extracts were subjected to phytochemical tests to determine their chemical constituents using standard methods as described by Richard and Cannell (1998) and Harborne (1984).

(1)Test for Carbohydrates

General Test (Molisch's Test)

Few drops of the Molisch's reagent were added to an aqueous solution of each of the extract, followed by addition of 1cm^3 of concentrated tetraoxosulphate(VI) acid (H_2SO_4) down the side of the test tube to form a layer. The mixture was allowed to stand for two minutes and made up to 5cm^3 with water. A red or dull violet coloration at the interface of the layer was taken as a positive test indicating the presence of carbohydrates (Brain and Turner, 1975).

Test for Reducing Sugars (Fehling's Test)

0.25g of each extract was dissolved in water and filtered. The filterate was heated with 5cm^3 of Fehling's A and B solutions, forming a red precipitate of cuprous oxide indicating the presence of reducing sugars (Sofowara, 1982).

Test for Combined Reducing Sugars.

0.2g of each of the plant extract was hydrolyzed by boiling with 5cm^3 of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. Few drops of Fehling's solutions A and B were added to each and heated on a water bath for 2 minutes. Formation of a red precipitate of cuprous oxide indicated the presence of combined reducing sugars (Trease and Evan, 1983).

(2)Test for Alkaloids

0.5g of the sample was defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 minutes with 5cm^3 of aqueous HCl on boiling water bath. The mixture was centrifuged for 10 minutes at 3000 rpm. 1 cm^3 of the filtrate was treated with few drops of Meyer's reagent and a second 1 cm^3 with Dragendroff's reagent and turbidity was observed (Harborne, 1984).

(3)Test for Anthraquinones

Free Anthraquinones

To each of the plant extract solution, 10cm^3 of benzene was added and shaken well; the content was then filtered and 5cm^3 of 10% ammonia solution was added to the filtrate. The presence of a pink, red or violet colour in the ammonical layer showed the presence of free anthraquinones (Trease and Evan, 1983).

Combined Anthraquinones

The extracts were boiled with 10cm^3 of 10% aqueous tetraoxosulphate(VI) acid (H_2SO_4) and then filtered. The filtrate was shaken with 5cm^3 of benzene (C_6H_6) and 10% ammonia solution (NH_4OH). A pink, red or violet colouration in the ammoniacal layer indicated the presence of combined anthraquinones (Trease and Evan, 1983).

(4)Test for Flavonoids

2g of crude powder was heated with 10cm^3 over a steam bath for 3 minutes. The mixture was filtered and 4cm^3 of the filtrate was shaken with 1 cm^3 of dilute. ammonia solution and yellow coloration was observed (Pithayanukul *et al*., 2007).

(5)Test for Saponins

About 0.5g of each extract was shaken vigorously with distilled water in a test tube, frothing (foaming) which persisted on warming indicated the presence of saponins (Edeoga *et al.*, 2005).

(6)Test for Cardiac Glycosides

Steroidal Nucleus (Salkowskii's Test)

0.5g of each extract was dissolved in $2cm^3$ chloroform followed by the addition of concentrated sulphuric acid (H_2SO_4) to form a layer. A reddish brown ring colour at the interface signified the presence of steroidal nucleus (i.e. aglycone portion of the glycoside) (Trease and Evan, 1983).

Keller-Killiani's Test for Digitalis Glycosides

About 0.5g of each extract was dissolved in $2cm^3$ of glacial acetic acid containing a drop of iron(III) chloride ($FeCl_3$) solution. To the mixture, $1cm^3$ of concentrated tetraoxosulphate(VI) acid (H_2SO_4) was added down the side of the test tube. And a reddish-brown ring obtained at the interface indicated the presence of a digitoxose sugar component characteristic of cardelonides (Brain and Turner, 1975).

(7)Test for Tannins

A small quantity of each extract was dissolved in water and heated on a water bath. Iron(III) chloride ($FeCl_3$) solution was added to the filtrate, a blue-black, green or blue-green precipitate was taken as a positive test signifying the presence of tannins (Sofowara, 1982).

(8)Test for Phlobatannins

A small portion of the extract were boiled with water and filtered. $5cm^3$ of 1% aqueous hydrochloric acid (HCl) was added to the filtrate. A red precipitate suggested the presence of phlobatannins (Sofowara, 1982).

(9)Test for Terpenes

A portion of the extract was diluted with distilled water and $1cm^3$ of acetic anhydride was added after which $1cm^3$ of concentrated tetraoxosulphate(VI) acid (H_2SO_4) down the walls of the test tube to form a layer underneath. The observance of the reddish-violet colour indicated the presence of terpenes (Trease and Evan (1983).

2.3 Antimicrobial Screening

Bacteria Culture

Five (5) bacterial isolates – *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus* were collected from the Microbiology Department of the Federal University of Technology, Minna, Nigeria. All cultures were checked for purity and maintained in nutrient broth slants.

Media Used

Two media were used for culturing the bacterial samples; nutrient agar and nutrient broth. 5g of nutrient agar was dissolved in $200cm^3$ of distilled water and sterilized. 6.5g of the nutrient broth powder was dissolved in $500cm^3$ of distilled water. The broth was pipetted into clean bottle containers, properly corked, wrapped in aluminum foil and sterilized in an autoclave.

Determination of Inhibitory Effects of Plant Extract on Bacteria

Inhibitory activities of the plant sample extracts were determined by inoculating the surface of already prepared nutrient agar with the isolates. Whatman's filter paper No.1 (6mm in diameter) was soaked in each of the extracts dried for about 2 minutes and placed on the surface of the inoculated plates. This was then incubated at 37^oC for 24 hours after which it was observed for any clear zone of inhibition. The observation of zone of inhibition was an indication that the plant extracts were able to hamper the growth of the organisms. The inhibitory actions of the extracts on the microbes were observed and tabulated.

3. Results and Discussion

3.1 Physical appearance of extracts

After extraction and concentration, the colour and texture of the extracts are stated in table 1 below:

Table 1: The physical appearance of extracts obtained from the leaves of *Tapinanthus dodoneifolios* (DC).

Solvent	Appearance
Methanol	Oily, deep blue-black sticky resin with a sharp odour. Very good yield.
Chloroform	Deep blue-black with a sharp odour. Poor yield
Water	Dark reddish brown with a pungent smell. Better yield than methanol and chloroform.

3.2 Phytochemical Contents the plant

Table 2: Results of phytochemical screening of the extracts of *Tapinanthus dodoneifolios* (DC) leaves

S/No	Chemical constituents	Test	Observation	Methanol extract	Chloroform extract	Water extract
1	Alkaloids	Harger's	Yellow ppt.	+	+	+
		Mayer's	Creamy ppt.	-	-	-
		Marqui's	Black colouration	+	+	+
2	Flavonoids	NaOH	Yellow ppt.	-	+	-
		Lead Acetate	Buff or creamy ppt.	+	-	+
		$FeCl_3$	Blue-violet colour	-	+	-
3	Tannins	$FeCl_3$	Blue-black or green colouration	++	-	+++
4	Saponins	Frothing or foaming	Persistent foam / froth	++	-	+++
	Carbohydrates:					
5	General	Molisch's	Red ring at interface	+	-	+++
6	Reducing sugars	Fehling's	Red ppt.	++	-	+++
7	Combined reducing sugars	Fehling's	Red ppt.	++	-	+++
	Anthraquinone					
8	Free anthraquinone	C_6H_6 /NH_3 solution	Pink, red or violet ppt.	-	-	-
9	Combined anthraquinone	H_2SO_4/ C_6H_6 /NH_3 solution	Pink, red or violet ppt.	-	-	-
10	Terpenes	Lieberman Burchard	Red/violet colouration	+	-	++
11	Phlobatannis	Filtrate + HCl_{aq}	Red precipitate	-	-	-
	Cardiac glycosides					
12	Steroidal Nucleus	Salkowskii's	Reddish ring	++	-	+++
13	Digitallis Glycosides	Keller-Killiani's	Reddish brown ring	+++	+	+++
14	Steroids	Terpenoid	Reddish ppt.	++	-	+++

Key:
+++ = Highly present, ++ = moderately present, + = minimally present, - = not present

3.3 Antimicrobial Screening Activity of Plant Extract

Table 3: The results of the sensitivity tests of the various extracts of *Tapinanthus dodoneifolios* (DC) leaves (in mm)

Organisms	Methanol	Water	Chloroform	Chloramphenicol	Ciprofloxacin
Escherichia coli	16	25	-	18	20
Salmonella typhi	20	18	-	22	28
Pseudomonas aeruginosa	-	21	15	20	16
Bacillus subtilis	-	-	-	13	28
Staphylococcus. Aureus	18	24	11	18	24

Key: - No Inhibition

Table 4: The Minimum Inhibition Concentration (MIC) of the leaves extracts of *Tapinanthus dodoneifolios* (DC) (in $mgcm^{-3}$)

Organisms	Methanol	Water	Chloroform	Chloramphenicol	Ciprofloxacin
Escherichia coli	6.25	6.25	-	6.25	6.25
Salmonella typhi	6.25	6.25	15.6	6.25	6.25
Pseudomonas aeruginosa	-	15.6	-	6.25	6.25
Bacillus subtilis	-	-	15.6	6.25	6.25
Staphylococcus. Aureus	6.25	6.25	15.6	6.25	6.24

Table 5: The Minimum Bactericidal Concentration (MCB) of *Tapinanthus dodoneifolios* (DC) leaves in $mgcm^{-3}$

Organisms	Methanol	Water	Chloroform	Chloramphenicol	Ciprofloxacin
Escherichia coli	25	25	-	25	25
Salmonella typhi	25	25	-	25	25
Pseudomonas aeruginosa	-	62.5	-	25	25
Bacillus subtilis	-	-	62.5	25	25
Staphylococcus aureus	25	25	62.5	25	25

3.4 Inhibitory Effects of the various extracts

Table 6: Activity of Plant Extract (*Tapinanthus dodoneifolius*) on selected microbes

Bacteria	Methanol Extract	Water Extract	Chloroform Extract	Chloramphenicol	Ciprofloxacin
Escherichia coli	Active	Active	Not Active	Active	Active
Salmonella typhi	Active	Active	Not Active	Active	Active
Pseudomonas aeruginosa	Not Active	Partial Activity	Not Active	Active	Active
Bacillus subtilis	Not Active	Not Active	Active at high concentrations	Active	Active
Staphylococcus aureus	Active	Active	Active at high concentrations	Active	Active

The results of the phytochemical screening of the various extracts indicated the presence of medicinally active constituents; alkanoids, flavonoids and glycosides in the methanol, chloroform and water fractions. Tanins, saponins, carbohydrates, terpenes and steroids were moderately and highly present in the methanol and water extracts respectively but were not detected in the chloroform extract. Phlobatanins, free and combined anthraquinones were not detected in all the three fractions. In general, the distribution of these phytochemicals in the three solvents was dependent upon their polarities and those of the extracting solvents (Tijjani *et al*., 2011).

The presence of tannins, saponins and alkaloids in these extracts explains the wide activities of the plant against the diseases caused by the test organisms in this study since they have been identified as highly antiviral and antibacterial agents (Tijjani *et al*., 2011). Also, tannins and flavonoids have been reported to be responsible for antidiarrhoeal activity while steroids have been said to have high anti-inflammatory and analgesics activities (Okoli, 2009). These thus could explain the role of *Tapinanthus dodoneifolius* as a good anti-inflammatory agent hence the justification of its local use in the treatment of wounds.

The methanolic and aqueous extracts had active inhibitory effects on *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* while the aqueous extract was only slightly active on the activity of *Pseudomonas aeruginosa*. The chloroform extract on the other hand, did not show any inhibitory effect on *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*; but partially inhibited the activity of *Bacillus subtilis* and *Staphylococcus aureus*. Although the antimicrobial activity of the aqueous extract of this plant covered the widest range of microorganisms because it probably had the highest number of phytochemicals, it was also not able to inhibit the activity of *Bacillus subtilis*. Interestingly however, in this study, it was observed that contrary to the general assertion made by many researchers as reported by Igbinoso *et al*. (2009) that aqueous extracts of plants show little or no antibacterial activities, the aqueous extract of this plant showed reasonable antimicrobial activities against the test organisms. This observation was also in line with the observation of Aina *et al*. (2010) on the antimicrobial activities of the aqueous extract of this plant.

Variations in the phytochemical components of the extracts were a clear pointer to the fact that the botanically active compounds in these media largely depend on the type of solvent and probably the extraction method(s) used. Although the traditional healers in this area use primarily water as the solvent of extraction, in this research we found that the plant extracts from both methanol and chloroform also exhibited varied antimicrobial activities which showed that such bioactive ingredients that might have not been extracted by water were extracted by the two organic solvents.

4. Conclusion

The test plant species, *Tapinanthus dodoneifolius* had the propensity of offering therapeutic actions against various infections especially those resulting from *Staphylococcus aureous*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and even *Bacillus subtilis*.

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