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Phytochemical Screening and Evaluation of Anti-typhoid Activity of Acalypha hispida Leaf Extracts Against Clinically Derived Isolates of Salmonella. typhi, Staphylococcus aureus and Escherishia coli From Patients Attending Aminu Kano Teaching Hospital

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Abstract

Phytochemical screening based on data from ethnopharmacological research is usually regarded as an efficient method for finding new anti-infective compounds in higher plants. Acalypha hispida leaves were percolated with 95 percent ethanol to obtain the crude extract. The plant`s ethanol extract was further fractionated using n-hexane, chloroform, ethyl acetate and methanol. The Acalypha hispida leaf extracts in ethanol had their phytochemical and antibacterial properties assessed. Studies on phytochemical screening found the presence of alkaloids, saponins, tannins, flavonoids and steroids. The disc diffusion method was used to assess antibacterial activity. Highest activity against S. aureus and S. typhi was demonstrated by the ethyl acetate fraction, with 14 and 8 mm, followed by the n-hexane fraction with 9 mm, and the methanol fraction with 8 and 8 mm all at 15 µg/disc, respectively. Meanwhile, methanol fraction with 10 mm on E. coli at the concentration of 15µg/disc, compared with the standard drug (chloramphenicol 30 µg/disc).Salmonella typhi and E. coli were found to be resistant to chloroform and n-hexane fractions. This proved that, Acalypha hispida leaves have ethnomedicinal activity against typhoid fever and some other gastrointestinal micro-organisms.

Keywords: *Acalypha hispida*, Phytochemical, Extract, Fraction, Disc diffusion, *S. typhi*

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Introduction

Due to reports that herbal medicines are safe and rarely caused side effects, especially when compared to conventional medications, there has been an increase in interest in the use of medicinal plants in poor nations in recent years. Therefore, it is preferable to look for new medications that have cheaper and better alternatives that come from plants. These plants` therapeutic properties were due to certain chemical compounds that had a clear physiological effect on the human body (Edeoga *et al*., 2005).

In some nations, such as China (Liu, 1987), Ethiopia (Desta, 1993), Argentina (Anesini and Perez, 1993) and Papua New Guinea, herbal remedies continue to play a significant role in the medical system (Nick *et al*., 1995). In Africa, medicinal plants have been widely used prior to the introduction of synthetic drugs (Kabir *et al*., 2005).

Nearly 80% of people in underdeveloped nations still receive their primary medical treatment through conventional medicine. At least 25% of the medications in modern pharmacopoeias come from plants, and many more are synthetic counterparts developed from plant-derived prototype chemicals (Desilva, 2005). In general, it is thought that using ethnopharmacological data to inform phytochemical research is a useful strategy for finding novel anti-infectives in higher plants (Kloucek *et al*., 2005).

Typhoid fever

Typhoid, often known as typhoid fever, is a widespread bacterial disease that can spread throughout the world by consuming food or water that has been tainted with an infected person`s faeces that contain the *Salmonella entrica, Serover Typhi bacterium*.

Other names for the illness include nervous fever, pathogenic fever, infantile remittent fever, gastric fever, and abdominal typhus. Typhoid`s name, which translate as “resembling typhus”, derives from the neuropsychiatric symptoms that both diseases share. Despite the similarities in their names, typhus and typhoid fever are separate

illnesses brought on by various bacterial species (Cunha, 2004).

Many typhoid carriers were imprisoned in an isolation ward around the turn of the 20th century in order to stop the spread of the disease. An estimated 16 to 33 million cases of typhoid fever were reported each year, and these individuals frequently suffered mental decline as a result of the conditions they endured. Between the ages of 5 and 19, children and young people are most likely to develop it (WHO, 2007). As of 2010, these incidents resulted in nearly 190,000 deaths, up from 137,000 in 1990 (Lozano *et al*., 2012). In the Democratic Republic of the Congo, an outbreak in 2004-2005 resulted in more than 42,000 illness and 214 fatalities (WHO, 2007).

Typhoid fever rates decreased in the majority of developed nations throughout the first half of the 20th century as a result of immunizations and improvements in public sanitation and hygiene. An important milestone in the management of typhoid fever in the United States in 1908 was the chlorination of drinking water. On the New Jersey Cite and its water supply, the first permanent treatment of drinking water in the United States took place. John L. Leal has received distinction for making the choice to construct the chlorination system (McGuire, 2013). George W. Fuller designed the chlorination facility (McGuire, 2013). In 1942, antibiotics were first used in chemical practice, which significantly decreased mortality rates. Typhoid fever is easily controlled and much more uncommon in wealthy countries than poor countries of around 5,700 cases per year (CDC, 2013).

Acalypha hispida (Euphobiaceae)

*Acalypha hispida*is also referred to as "Akalifa" in Hausa, as well as red "hot cat`s tail,""Philippines Medusa" and "fox tail" in English. The chenille plant, *Acalypha hispida*, is a flowering shrub that is a member of the genus *Acalypha* and the subfamily *Acalyphinae* of the family Euphorbiaceae.

Due to their dieocious nature, the plants have separate male and female members. The female plant produces clusters of pistillates flowers that

range in color from deep purple to bright red and grow along catkins; these characteristics are the main causes for the plant`s common name, "red-hot cat`s tail". With potted plants having the smallest growth potential, it can reach heights of five to twelve feet (1.8 to 3.7m) and widths of 3 to 6 feet (0.9 to 1.8m). The tropics of Africa, America, and Asia are where the plant *Acalypha hispida* naturally occurs. Considering the type and color of the plant`s blossoms, domestication is the only accurate description. Both cuttings and seeds can be used to cultivate it. It may be kept as a houseplant or as an outdoor plant. However, caution should be used when growing it because all components of the plant are lethal to animals if consumed.

According to reports, *Acalypha hispida* alcohol extracts are biologically effective against *S. aureus, E coli, P. aeruginosa,* and *S. typhi* (Okorondu *et al*., 2009). Additionally, it has been demonstrated that *A. hispida* leaf extract has antifungal properties (Ejechi and Soucey, 1999). More of these biological processes have been linked to its inherent pro-oxidant lowering potential (Han *et al*., 2007). Leprosy is traditionally treated with a poultice made of leaves. An infusion of the leaves and blossoms is taken internally as a laxative and diuretic in cases of gonorrhea and applied as an emollient to wounds and sores. Asthma relief and expectorant properties of the bark root are thought to exist. The blooms are used to treat diarrhea and dysentery, while the leaves are astringent (Bokshi *et al*., 2012).

The ethanolic extract of *Acalypha hispida* underwent phytochemical analysis, and the results showed the presence of reducing sugar, glycoside, steroid, flavonoid, and saponin. The leaf extracts of *A. hispida* in both aqueous and methanolic form contained phenolics, flavonoids, glycosides, steroids, saponins, phlobatannins, and hydroxyanthraquinones (Iniaghi *et al*., 2009). The plant was used to produce gallic acid, corilagin, cycloartane-type triterpenoids, and flavonoids like quercetin and kaempferol derivatives (Adesina *et al*., 2000)

.Methodology

Plant Collection

At the Musa Adamu garden near the school of management studies` new location on Yahaya Gusau Road in Kano State`s Gwale local government area, fresh leaves of *A. hispida* (botanical name) was obtained. Identified at the Bayero University Kano herbarium, where a voucher number of BUKHAN 253 was deposited by mal. Baha`uddeen Sa`id Adam. The sample was cleaned, allowed to dry in the shade, and then used a mortar and pestle to be ground into powder.

Extraction

Acalypha hispida leaf powder weighing 200g was percolated in 950 mL of absolute ethanol for three days at room temperature. Using rotary evaporator (R110) at 40ºC, the extract was filtered and concentrated to lessen the amount of solvent in the mixture. The resultant crude extract was weighed and given the name "ethanolic extract."

Fractionation

In order of increasing polarity, n-hexane, chloroform, ethyl acetate, and aqueous methanol were used as different solvents to fractionate the crude ethanol extract of *A. hispida* into various fractions (Ingle *et al*., 2017).

The crude extract was dissolved using 250cm^3 of water before being added to a separating funnel, agitated, and then allowed to settle. N-hexane, the least polar solvent, was also added, and 250cm^3 of it was shaken. As the material settled, the separating funnel`s bottom opened, allowing the aqueous layer to be removed. To obtain the n-hexane fraction, the organic layer in the separating funnel was emptied into a clean beaker (Ingle *et al*., 2017). N-hexane was once more added in equal amount (250cm^3), shaken, and separated. After adding n-hexane and shaking, the addition proceeded until no appreciable amount of extract appeared to flow into the n-hexane fraction (Ingle *et al*., 2017).

Ingle *et al*., used a similar technique on chloroform, ethyl acetate, and methanol to obtain the correspondingly labeled chloroform, ethyl acetate, and methanol fractions.

Phytochemical Screening

The phytochemical screening methodologies carried out were modified from the earlier work done on other different plants analysis (Odebiyi and Sofowora, 1979). It was done to determine whether alkaloids, saponins, tannins, flavonoids and steroids were present.

Test for Alkaloids

A test tube containing the extract (2 cm^3), 0.2 cm^3 of dilute HCl was added, and then filled with 1cm^3 0f Dragendorff`s reagent. Alkaloids are present as shown by the Orange yellow tint (Harbone, 1998).

Test for Saponins

20 cm^3 of distilled water were added to the extract (1 cm^3) in a test tube for 15 minutes, it was shaken with hands. On the test tube`s top, a layer of form was created. This suggests that saponins are present (Harbone, 1998).

Test for Tannins

The concentrated sulphuric acid (1 cm^3) was carefully added to the solution at the side of the test tube after the extract (3 cm^3) had been carefully put in the test tube and diluted with chloroform, acetic anhydride, and chloroform (1 cm^3). The formation of a green color indicates the presence of tannins (Harbone, 1998).

Test for Flavonoids

To 1 cm^3 of the extract, 0.2 cm^3 of aqueous sodium hydroxide was added. The presence of flavonoids was suggested by the presence of a bright yellow color (Harbone, 1998).

Test for Steroids

An equal volume of concentrated sulphuric acid was added to the test tube by the sides after the crude plant extract (1 mg) had been dissolved in 10 cm^3 chloroform. The test tube`s upper layer turned red and the acid layer displayed yellow with green fluorescence, indicating the presence of steroids (Harbone, 1998).

Antibacterial Test

Using the disc diffusion method, *A. hispida`s* antibacterial activity was evaluated (Bauer *et al*., 1996). This procedure involved dissolving specified amounts of the test samples in specific volumes of solvent to create solutions with the appropriate concentrations (µg/disc).

Isolation of Organisms

Clinical isolates from Kano`s Aminu Kano Teaching Hospital, which were confirmed using biochemical test at the microbiology department of Bayero University Kano were used as the test organisms, and then put through an antibacterial test.

Preparation of Sensitivity Discs

Paper punches were used to cut out Whatman No.1 filter paper discs (6mm in diameter), which were then put in bijou bottles. They were sterilized using autoclave for 15 minutes at 121ºC to disinfect them and allowed to cool (Yusha`u, 2011).

Preparation of Stock Solution

In 1mL of dimethyl sulphoxide (DMSO), 0.06g of each extracts was dissolved. To get a 60µg/disc concentration, half (0.5mL) of the extract was added to fifty sterile discs in bijou bottles. Half (0.5mL) of the DMSO was added to the remaining stock solution to make 1mL, and then another 0.5mL was taken and put into a bottle with fifty filter paper discs and labeled as 30µg/disc. The remaining 0.5mL of DMSO was then added, and a final 0.5mL was taken and put into a bottle with fifty filter paper discs and labeled as 15µg/disc. The method was used to create concentrations of 15, 30, and 60µg/disc, with each disc able to

absorb 0.01mL of the solution (Yusha`u, 2011).

Bioassay Procedure

Disc Diffusion Test:

Standard inocula of the isolate were swabbed on the surface of prepared and hardened Mueller Hinton agar in various petri-dishes for the disc diffusion test. The surface of the infected media was periodically covered with the produced discs of the extract fractions (n-hexane, chloroform, ethyl acetate and methanol). The plates were incubated at 37ºC for 18-24 hours before zones of inhibition were detected and measured (NCCLs, 2002).

Results

Table 1: Extraction

Plant extracts	Weight (g)	Texture	Colour
Crude extract	12.28	Gummy	Dark brown
n-hexane	1.52	Oily	Dark green
Chloroform	0.40	Oily	Dark green
Ethyl acetate	0.54	Powder	Light brown
Methanol	8.16	Gummy	Dark brown

Table 2: Phytochemical Screening

Fractions	Alkaloids	Saponins	Tannins	Flavonoids	Steroids
n-hexane	+	-	-	+	-
Chloroform	-	-	-	+	-
Ethyl acetate	+	+	+	+	+
Methanol	-	+	+	-	-

KEY: + = Present - = Absent

Antityphoid Test Results

Table 1: n-hexane Fraction

Test organism/Concentration	60µg/disc (mm)	30µg/disc (mm)	15µg/disc (mm)	CHL 30µg/disc (mm)
S. typhi	12	0	0	15
E. coli	0	0	0	25
S. aureus	14	12	9	18

CHL = Chloramphenicol

Table 2: Chloroform Fraction

Test organism/Concentration	60µg/disc (mm)	30µg/disc (mm)	15µg/disc (mm)	CHL 30µg/disc (mm)
S. typhi	0	0	0	15
E. coli	8	0	0	25
S. aureus	10	0	0	18

CHL = Chloramphenicol

Table 3: Ethyl acetate Fraction

Test organism/Concentration	60µg/disc (mm)	30µg/disc (mm)	15µg/disc (mm)	CHL 30µg/disc (mm)
S. typhi	14	12	8	15
E. coli	12	10	0	25
S. aureus	16	15	14	18

Table 4: Methanol Fraction

Test organism/Concentration	60µg/disc (mm)	30µg/disc (mm)	15µg/disc (mm)	CHL 30µg/disc (mm)
S. typhi	11	10	8	15
E. coli	11	10	7	25
S. aureus	16	14	8	18

CHL = Chloramphenicol

Discussion

The plant under study, *A. hispida,* was gathered based on its ethnomedical applications for treating typhoid fever. According to phytochemical analysis of the extract fractions, alkaloids, saponins, tannins, flavonoids, and steroids were all present. Only flavonoids were visible in the chloroform fraction, while alkaloids and saponins were visible in the n-hexane fraction. Alkaloids, saponins, tannins, flavonoids, and steroids were also found in the ethyl acetate fraction, whereas only saponins and tannins were found in the methanol fraction.

The ethyl acetate fraction had the strongest activity in the antityphoid bioassay of the extract fractions, with zones of inhibition measuring 14, 12, and 8mm at disc concentrations of 60, 30, and 15µg/disc respectively. While at a concentration of 60µg/disc, the n-hexane fraction was found to have the lowest activity, with just a 12mm zone of inhibition. Furthermore, it was discovered that the chloroform fraction was resistant at all concentrations. With a 12mm zone of inhibition at a concentration of 30µg/disc, ethyl acetate was discovered to be the most active fraction when compared to the concentration of the standard medication, chloramphenicol.

Conclusion

The n-hexane, ethyl acetate, and methanol fractions from the leaves of *Acalypha hispida* were found to have antityphoid activity. The test also revealed significant ethyl acetate activity. The presence of flavonoids, saponins, and steroids that were discovered in phytochemical studies is likely what gives *Acalypha hispida* its antibacterial properties. Therefore, the findings are consistent with the plant`s historical use as a typhoid fever cure. It is possible that the *Acalypha hispida* will have high activity at higher concentrations. Further research is therefore advised at a higher concentration.

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