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Production, Evaluation and Packaging of Zoginsic Tea for the Management of Diabetics, Renal Dysfunction and Urinary Tract Infections.

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Abstract

The use of medicinal plants in traditional medicine is an age long practice and the quest for novel approaches to remedying the problem of microbial drug resistance is of paramount importance in the pharmaceutical industries. This study is about the production, packaging, phytochemical screening , synergistic cidal effect, anti- diabetics, renal and urinary tract protective potential of Zoginsic tea a composite of *Hibiscus sabdariffa* (zobo flower), *Zingiber officinale* (Ginger) and *Stigma maydis* (Corn silk).The phytochemical screening was carried out using standard methods. The antibacterial activity of the tea extract was determined using the agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tea extract on the test isolates were determined using the micro broth dilution method. The Ant- diabetic study was carried out using a total of twenty-five (25) adult male Wistar rats (8 weeks old, 160 ± 5 g) and for renal and urinary tract protective potential of Zoginsic tea 42 patients (16 male, 26 female) with age of 25.31 ± 4.57(mean ±SD) was used. The phytochemical analysis showed that alkaloids, phenol, flavonoids, tannin, saponin, glycosides and anthraquinones were present in varying concentrations of the different extracts. The methanol extracts of the zoginsic tea possesses more antimicrobial activity (18- 28.5mm) in a concentration dependent manner than aqueous extract (10 – 27mm). The MIC of the composite plant extracts on the test organisms ranged from 12.5 – 25mg/mL while the MBC ranged from 6.25 – 12.5 mg/mL. The anti-diabetic effect of the tea show the potency of the tea as an ant-diabetic therapy .Administration of aqueous extract of zogisic tea to UTI patients result in significant $P<0.05$ decrease in UTI symptoms after 5 days, 10 days and 20 days respectively when compared to baseline values. The research reveals that *zoginsic* tea extract has a broad spectrum of activity against the test organisms and therefore, should serve as a cheap source of antibacterial agent, diabetes, renal dysfunction and urinary tract infection management

Key words: Zoginsic Tea, Management , Diabetics, Renal dysfunction and Urinary Tract Infections.

Introduction

Urinary tract infection (UTI) and diabetes is the second most common infections presentation in community practice; worldwide about 150 million people were diagnosed with UTI each year and diabetes . For better management and prognosis, it is mandatory to investigate the possible

site of infection, occurrence of re-infection, or relapse, treatment failure in addition to pathogenesis and risk factors (Najar, etal., 2009).UTI may involve only the lower urinary tract or may involve both the upper and lower tract. The term “cystitis” has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and suprapubic pain . Pyuria is an expected accompaniment of significant bacteruria. The absence of pyuria is considered useful in excluding UTI (Saadeh, *et al*,. 2011). The increasing prevalence of antimicrobial resistance is a major health problem; many bacterial species including E.coli are showing an increasing resistance to antibiotics. Multidrug resistance among E.coli isolates have been reported from many parts of world, and these rates of resistance to antibiotics differ from nation to nations .Through, the world there are numerous reports for the use of herbal treatment of UTI; one of these most common herbal agents are corn silk and ginger and hibiscus sabdarriffa which are component of zoginsic tea. With potent phytochemical whose synergic ability is the trust of this research in the management of diabetes renal dysfunction and urinary tract infections.

Materials and Method

Study Area

The study was conducted at Federal Polytechnic, Auchi, Department of Biological and Physical Laboratory Sciences, Auchi is located in the northern part of Edo State within the coordinates of latitude 07^{0}04’N and longitude 06^{O}16’E. It is situated in the south-south geographical zone of Nigeria with a population of over 500,000 people according to the 2015 population census. It is approximately one hundred and thirty kilometer (130 Km) away from Benin City, the capital of Edo State. Auchi is the headquarters of Etsako West L.G.A. and has witnessed territorial development owing to rural-urban migration. It is bounded to the North by Jattu, to the south by Aviele, to the east by Iyakpi and to the west by Owan Local Government Area. It is also the seat of the Federal Polytechnic, Auchi, Edo State, Nigeria.

Sample Collection

Red calyces of *Hibiscus sabdariffa* (zobo flower), rhizome *Zingiber officinale* (Ginger) and *Stigma maydis* (Corn silk) were purchased fresh from Uchi Market, Auchi, Edo State, Nigeria.

Production of Zoginsic Tea

Equal weight (20g) of the respective plant sample: *Hibiscus sabdariffa* (zobo flower), rhizome of *Zingiber officinale* (Ginger) and *Stigma maydis* (Corn silk) was composed to obtain the zoginsic tea after subjection to clinical drying, milling and other preparatory conditions.

Packaging of the Zoginsic Tea

The composite tea (zonginsic) after production was firstly package in a tea bag and ten sachets each where final package in a cellulose water resistance container designed and invented by the Art and design department of Auchi polytechnic , Auchi ‘

Evaluation of the phytochemical content of the zoginsic tea.

The plant samples were screened for the following compounds: alkaloids, phenol, terpenoids, saponnins, tannins flavonoids glycosides, steroids, and anthraquinone, by the method described by Sofowera (1993).

Preparation of Methanolic and Aqueous Extracts

The Plant extracts used in this study were prepared according to the protocol described by Akinnibosun (2009), with modification.

Preparation of Aqueous Extract of Zoginsic tea

20g of the Zoginsic tea was dissolved in 100mL of distilled water for 24 hours (stirred every 6 hours). Using a Whatman’s no.1 filter paper, the resulting mixture was filtered to obtain a solid-free solution. Rotary evaporator and water bath was used to evaporate and concentrate the filtrate to dryness and the resulting extract was collected using a sterile universal bottle. It was then stored at 4^0C in a refrigerator for further investigation.

Preparation of Methanol Extract

20g of zoginsic tea was suspended in 100 mL of 95% methanol and stirred every 6 hours for 24 hours. Whatman’s no.1 filter paper was used to separate the mixture so as to obtain a solid-free solution. The filtrate was evaporated to dryness with the aid of a rotary evaporator and the resulting extracts were store at 4^OC for further analysis.

Extract dilution

After the extracts were prepared following the protocol described by Akinnibasun with modification, the aqueous and methanol extracts were reconstituted using sterile distilled water to obtain the following concentrations: 200, 100, 50, 25, 12.5 and 6.25 mg/mL (Akinnibosun, 2009).

Clinical bacterial Isolates

The bacterial isolates comprising of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus*, were obtained from the Laboratory section of the Federal Polytechnic Cottage Hospital, Auchi, Edo State, Nigeria. Standard Identification methods including cultural, morphological, and biochemical tests, were employed to confirm the identity of the isolates (John *et al.*, 2000). Agar slants were used to store the bacterial isolates at 4^{O}C until further use.

Preparation of inoculations and Assays of Antibacterial Activities.

The inoculation of the bacteria was done by pouring method and the antibacterial susceptibility of the tea extracts was carried out by Agar Well diffusion method following the protocol described by Unegbu *et al*. (2020

Agar-well diffusion technique was used to carry out the antibacterial susceptibility of the plant extracts in comparison with standard antibiotic gentamicin (20mg/ml) in vitro on the isolates according to the methods of National Committee for Clinical Laboratory Standards (NCCLS, 2007). Pure culture of the bacteria was grown on nutrient agar. Three colonies of each organism were pick into the Mueller Hinton broth (Oxoid, England), incubated for 4 hours at 37^{O}C, and diluted with sterile saline to a density visually equivalent to the MacFarland Standard. Using a sterile 6-mm diameter cork borer, six (6) wells were cut in the agar to which the tea extracts was added, the standard drug, gentamicin (GEN, 20mg/mL) and sterile water separately, which served as the positive and negative controls, respectively. The plates were incubated at 37^{O}C for 24 hours. The zones of inhibition were then measured with aid of a meter rule.

Determination of Minimum Inhibitory concentration (MICS)

The minimum inhibitory concentrations of the tea extracts on the sensitive organisms were determined by the broth dilution method as described by Unegbu *et al*.(2020).

5mL of the various tea extracts were transferred into different test tubes, 5mL of nutrient broth each was added and serially diluted out to various concentrations ranging from 200 to 12.5 mg/ml. A loop full of each test bacteria was inoculated into each of the test tube and subsequently incubated for 24 hours at 37^{O}C. The lowest concentration of the tea extracts that inhibited the growth of the test organisms was the MIC (Cheesebrough, 2002).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the tea extracts was carried out according to the protocol described by Unegbu *et al*. (2020), with modification.

ImL bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes and were sub cultured onto nutrient agar plate. They were subsequently incubated at 37^{O}C for 24 hours. This was obtained by streaking out the samples from the MIC tubes that showed no visible growth on nutrient agar plates. The least concentration of the sample that showed no growth was noted and recorded as the minimum bactericidal concentration (Cheesebrough, 2002).

Ant diabetic study

Animal **and ethics statement**

A total of twenty-five (25) adult male Wistar rats (8 weeks old, 160 ± 5 g) was obtained from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma and used for this study. The animals were hygienically housed in plastic cages placed in a well-ventilated vivarium with natural photoperiod of 12-hr light and dark cycle. They were fed with rat chow and given drinking water *ad libitum* during one week of acclimatization before the commencement of the experiment. All the animals received human care as indicated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Animal Use and Care Committee (AUCC). All the protocols in this experiment were approved by the, National Health Research Ethic Committee, Nigeria.

Induction of Diabetes

Diabetes was induced following the method of **Umar (2016).** All experimental animals used were male albino mice.The Wister rat were fasted, however, for 12 hours before experiment..

Experimental design

Diabetes was induced by a single intraperitoneal injection of 120mg/kg body weight alloxan monohydrate freshly dissolved in fixed saline 0.9% physiological saline immediately before used to overnight fasted Wister rats . Seven days after, animals with fasting blood glucose level ≥146

mg/dL or more were considered diabetic and employed in the study. The diabetic Wister rats were then grouped into 5 groups of five rat each as follows

Group I: Served as positive control and received glibenclamide (3 ml/kg body weight)

GroupII: Received Methanol extract at 250 mg/kg bodyweight

Group III: Received Methanol extract at 500 mg/kg body weight

Group IV: Received Methanol extract at 750 mg/kg body weight

Group V**:** Served as negative control receiving physiological saline (10 ml/kg body weight)

The animals were treated once and blood glucose concentrations were measured at 0, 24, 48, 72 and 96 hours. Blood samples were taken by a snip-cut at the tip of the tail and blood glucose levels were measured with a glucometer (a ONE TOUCH Ultra easy blood glucose monitoring system, Life Scan Europe Division of Cilag GmbH international 6300 Zug Switzerland).

Investigation of the Potency of Zogisic tea on U.T.I. Patients

This Prospective randomized clinical trial was carried out on 42 patients (16 male, 26 female) with age of 25.31 ± 4.57(mean ±SD); who attended outpatient clinic in Auchi Polytechnic Cottage Hospital, from November 2021-March 2022. The study was approved by scientific and ethical committee of the polytechnic and consent was taken from all patients. The protocol criteria were defined and explained to the patients. Pregnant patients with chronic diseases were exempted. For all patients, UTI was confirmed according to standard procedure described by Ahmed et al. (2012). All of the patients were followed up after 5days, 10days and 20 days from starting course of treatment with aqueous extract of Zogisic tea.

Result

Phytochemical screening

The phytochemical analysis of the various plants extract used in this study shows the presence of alkloids, saponins, phenol, flavonoids, terpenoids, tannins and anthraquinones in vary concentrations in the methanol and aqueous solution as shown in Table 1.

Table I: Phytochemical composition of Zoginsic tea

Phytochemical components	Mehanol extracts	Aqueous extracts
Alkaloids	+++	-
Flavonoids	+++	-
Anthraquinolone	++	+
Saponins	-	++
Glycoside	++	++
Steroids	+	-
Phenol	+++	++
Terpenoids	++	+
Tannins	++	+

-, absence; +, slightly present; ++, moderately present; +++, highly present.

Antidiabetic effect of methanol extracts of zoginsic tea extract in alloxan- induced diabetic wistar.

The values of blood glucose levels are given as mean ± SEM (n = 5), and the values indicated significant ($P<0.05$) of anti-diabetic effect with respect to both positive, negative controls and in comparison with the extract treatment Differences between groups were also considered significant at 5% level of significance i.e. $P \leq 0.05$ table 2 below.

Table 2: Anti-diabetic Effect of methanol ethanol extracts of zoginsic tea extract in alloxan-induced diabetic wistar.

Test materials	group	**Blood glucose levels in (mmol/L) sampling time in hours**				
		0	**24**	**48**	**72**	**96**
Glibenclamide (positive control)	Group I	19.5 ±1.6	17.3± 3.0	16.5±1.4	15.4± 2.7	14.2±2. 1

Group								

250 mg/kg	Group 2	17.1 ±4.5	16.4± 2.5	14.8±2.0	13.3± 2.7	13.9±4. 2									
500 mg/kg	Group 3	15.8 ±2.1	14.2± 1.3	13.8±1.4	12.4± 2.2	13.5±1. 1									
750 mg/kg	Group 4	13.4 ±0.7	14.0± 1.3	12’9.6± 0.8	13.3± 2.1	13.3±1. 5									
Physiological saline (negative control)	Group 5	20.5 ±1.2	19.7± 3.0	18.2±2.1	19.6± 1.3	18.2±1. 3									

Values of blood glucose levels are given as mean ± SEM (n = 5), and the values indicated significant ($P<0.05$) anti-diabetic effect with respect to both positive and negative controls. Differences between groups were also considered significant at 5% level of significance i.e. $P \leq 0.05$

Table 3: Result of antibacterial activities of methanol and aqueous extracts of zoginsic tea on bacterial isolates.

Extracts concentrations (zone diameter of inhibition in mm)								
Isolates	200	100	50	25	12.5	+c	-c	Extracts
E. coli	20	18	16	14	12	18	0	AE
	27	25	2.5	21	19	20	0	ME
Staph. aureus	17	15	13.5	12	10	16	0	AE
	26.5	24	22	20	18	18	0	ME
P. aeruginosa	*27*	*25*	*23*	*21.5*	*19*	*17*	*0*	*AE*
	28.5	26	25	23	20	18	0	ME
B. cereus	25	23	21.5	20	18	18	0	AE
	27	25.5	24	22	20	17	0	ME

AE, Aqueous Extract; ME, Methanol Extracts; +c, Positive control (gentamicin, 20mg/mL); -c, negative control (distilled water).

Table 4: Minimum Inhibitory and bactericidal concentrations of zoginsic tea on bacterial isolates.

Concentration of Extract (mg/mL)			
Isolates	**MIC (mg/mL)**	**MBC (mg/mL)**	**Extracts**
E. coli	25	25	AE
	12.5	6.5	ME
S. aureus	25	25	AE
	12.5	12.5	ME
P. aeruginosa	25	25	AE
	12.5	6.5	ME
B. cereus	25	25	AE
	12.5	12.5	ME

AE, Aqueous extract (distilled water); ME, Methanol extract; MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration.

The Potency of Zogisic tea on U.T.I. Patients

Administration of aqueous extract of zogisic tea to UTI patients result in significant P<0.05 decrease in UTI symptoms after 5 days, 10 days and 20 days respectively when compared to baseline values. Result as in table 3 showed that blood urea and serum creatinine were not significantly differ in patients with UTI when compared to base line baseline , nor after starting the treatment course; at the same time, no any side effect was reported during the course of treatment, indicating the safety of aqueous extract of zoginsic tea on UTI.

Table 5: Time course incidence of UTI symptoms

Time (days)	Suprapubic pain	Urgency	Frequency	Dysuria
0	78 (78.57%	35 (83.33%)	35 (83.33%	36(76.19%
5	10 (11.9%)	10 (23.8%)	11	5
10	5 (2.38%)	2 (4.76%)	3	2
20	0 (0.0%)	0 (0.0%)	1	1

Results represent percent of total, * significant change P 0.05.

Table6 : Time course change in renal function test of UTI patient

Time(days)	Bl.Ureammol/L	S.Cr. mol/
0	4.46 0.21	76±3.2
5	3.4±0.22	66±1.4
10	2.3±1.4	56±2.4
20	2.1±3.4	50±1.8

Results represent mean SD . blood urea and serum creatinine

Discussion

The phytochemical analysis of *zoginsic* tea reveals the presence of cardiac glycoside, saponnin, alkaloids, flavonoids, terpenoids, phenols, anthraquinones and tannins. These phytochemicals are determinants of the antimicrobial activity and therapeutic significant of medicinal plants used in folk medicine. The presence of cardiac glycoside in the sample extracts give credence to their popular use in the treatment of hypertension. Cardiac glycosides are cardio active compounds belonging to triterpenoids class of compounds. Their clinical effects in cases of congestive heart failure are to increase the force of Myocardial contraction (Brain *et al*., 1985). They exert their hypotensive effect by inhibiting Na^{+}-K^{+} ATPases. Saponins are glycoside of both triterpenoids and steroids having hypotensive and cardiac depressant properties. Saponins bind to cholesterol to form insoluble complexes; dietary saponins in the gut of monogastric combine with endogenous cholesterol excreted via the bile. This prevent cholesterol re-absorption and results in a reduction of serum cholesterol (Cheeke, 1971). Saponins have been found to be potentially useful for the treatment of hypercholesterolemia which suggest that saponins might be acting by interfering with intestinal absorption of cholesterol (Malinow *et al*., 1977).

Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Chabner and Horwit, 1990; Noble, 1990). Alkaloids also interfere with cell division, hence the presence of alkaloids in the various extracts could account for the antimicrobial activity of zobo flower, ginger and corn silk used in this study. This is in keeping with the findings of Che-wonarin *et al.* (1999) that isolated extract of alkaloid from *Hibiscus sabdariffa* demonstrated ability to prevent mutagenesis. Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown

to exert potent antioxidant activity against the superoxide radical. Its consumption has been demonstrated not to be associated with coronary heart disease mortality (Hertog *et al*., 1993).

Antibacterial effects of these tea extracts against *Staphylococcus aureus, Escherichia coli, Bacillus cereus and Pseudomonas aeruginosa* suggest that they may possess remarkable therapeutic effect in the treatment of gastrointestinal infection and diarrhea in man and skin diseases (Roger *et al*., 1990).

From the experiment, the absence and varying concentrations of most of the phytochemicals might be due to differences in the polarity of the solvents, as the types of solvent used determined the kind of biologically active compounds that can be extracted from the plant (Tiwari *et al.*,2011).

It has been reported that different solvents have different extraction capabilities (Ashok *et al.*,2014). Ashok *et al.* (2014) reported that the best way to extract broad-spectrum antimicrobial compound from plant is by the use of methanol solvents. The differences observed between antibacterial activities of the extracts could be explained by their abilities to dissolve in different solvents (Ashok *et al.*, 2014).

Results from this study shows that the methanol extracts of the zoginsic tea inhibited the growth of the test organisms than the aqueous extract in a concentration – dependent manner. The variation in the antibacterial activities is due to difference in the quantity of compounds present in the tea extracts (Ezeifeka, 2004). Similar result was discovered in the work of Ewansiha (2014).

The result also showed a greater zone of inhibition produced by the methanolic extracts of the zoginsic tea (18 – 28.5mm). Zoginsic tea a composite of *Hibiscus calyces* – ginger – corn silk mixture could serve a better and alternative drug to treat bacterial infection compared to most conventional antibiotics used presently. Similar result was discovered in the work of Fullerton *et al.* (2011).

The MIC obtained show that different concentrations were effective against the test organisms. The generally low MIC and MBC values of methanol extracts against the test organisms are an indication of their antibacterial potential (Elzein *et al*., 2018).

The use of herbal medicine in the treatment of disease in general and specially UTI is not a new approach; it has been reported that the Ebers papyrus from ancient Egypt recommended herbal treatment to enhance urinary symptoms without providing insight into pathological mechanisms

(*Nickel*, 2005). Urinary tract infection (UTI), acute and chronic, can be effectively treated with herbal medicine. It has been shown that, there are two strategies which are essential in utilizing herbal medicine; the choice of herb depending on its herbal action, and the appropriate therapeutic dosing strategies that will determine the effectiveness of herbal treatment and prevent the need to intervene with antibiotics (*Dipasquale*,2008). It has been found that medicinal plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids; these metabolites have been found in vitro to have antimicrobial properties (*AlamSher*,2009). Many plants have been evaluated not only for their inherent antimicrobial activity, but also for their action as a resistance-modifying agent (*Darwish*, 2010). It has been believed that certain medicinal plants can prevent the recurrence of UTI; examples of such are corn silk, garlic which is major component of this tea. The mechanisms of action are believed to include stimulation of the immune response, inherent antimicrobial activity, also for their action as a resistance-modifying agent(*Darwish*, 2010).Other mechanism includes stimulation of the immune response, change in urinary PH (Reid, 1999), and prevention of growth and adhesion of pathogens (*Lüthje*, 2011). The work is corroborated by the works (*Hannan*,2010 and *Peng*, *et al.*,2008) that many medicinal plants have been used in the treatment of UTI because of their anti-inflammatory effect All the above mentioned mechanisms may explanation for the results obtained in this study; although diuretic and uricosuric properties have traditionally been attributed to components of *zoginsic tea* .

Conclusion and Recommended

Zoginsic tea (hibiscus calyces – ginger – corn silk) extracts mixture was found to have more antimicrobial effect than most conventional antibiotics used presently. The secondary metabolites present in the plants extract, such as phenols, flavonoids, glycosides, tannins, saponins, terpenoids, alkaloids, and anthraquinone were responsible for inhibition of the test organisms in this present study and could also justify their use as antimicrobial agents in traditional medicine

In conclusion, our results showed that administration of aqueous extract of zoginsic tea significantly reduce the symptoms in patient with UTI in addition it does not manifest any negative impact on the renal chemical profile like blood urea and serum creatinine hence the

consumption of zoginsic tea is recommended for the management of hypertension , renal dysfunction and neurodegeneration.

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