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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING AND NUTRITIONAL QUALITIES OF FICUS SUR (FORSSK)

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Abstract:
The preliminary study of the leaf of Ficus Sur (Forssk) was conducted to ascertain its medicinal and nutritional qualities. Phytochemical analysis and antimicrobial activity were conducted. Micro elements and vitamins B and C were also determined by atomic absorption spectrophotometry and titrimetry respectively. Results of the analysis revealed the presence of iron, copper, magnesium and manganese as well as vitamin C and B at various levels. The leaf was found to show antimicrobial activity against many bacteria such as Enterobacter aerogens, Bacillus species, Pseudomonas pyocyania and Staphylococcus aureus. Antifungal activity against Aspergillus niger and Aspergillus flavus was also established. The phytochemical study indicated the presence of flavonoids, saponins, alkaloids, tannins, proteins, reducing sugar, fats and oil and carbohydrate. The leaf extract of this plant can therefore satisfactorily cure diseases like anemia, malnutrition, cold and supportive infections like boil, skin pustules, conjunctivitis, septicaemia, intestinal diseases, food poisoning, surgical wounds and burns, acute bacterial endocarditis and bacterimia.

Keywords: Phytochemical; Antimicrobial; Nutritional.

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1. Introduction

Plant products have been used by ethnic tribes of Nigeria from time immemorial for treatment of various ailments and protection against disease vectors. Some of the products have also served as sources of drugs for pharmaceutical industries.

A medicinal plant is any plant which contains in any of its organs substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1982). Apart from their efficacy, it is very important to mention their little or no side effects in the treatment of diseases because they act as foods and medicines. In the treatment of hypertension for instance, the herb is used first to lower the blood pressure to clean the arteries, to slow down and regulate the heartbeat, to improve the blood circulation and to relax the mind. Unlike the conventional ones that will dilute the arteries or the veins until they reach their maximum elastic point which may suddenly burst and cause vascular accident causing stroke or death. There are

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only a few or no synthetic drugs that will combine all the functions listed above which the herb will perform (Kafaru, 1994).

Ficus Sur (Forssk) formerly called Ficus capensis, has common names among the tribes in Nigeria {Akankoro in Igbo, opoto in Yoruba and dullu in Hausa). The plant belongs io the family Moraceae. They are found mostly in the tropics and warm temperate regions (Hutchinson and Daniel,1967, Lawal etal.,2012 and Solomon-wisdom,2011).

Like other Ficus species Ficus Sur (Forssk) is a tree which is 6-9metres high and 0.6-12m in girth. It is epiphytic with milky white latex. The latex is sticky and locally used as gum. The leaves alternate and measure 2.5 to 15 cm long. When fresh, they are greenish but when dry they look very light green about gray in color (Keay, 1989).

Ficus Sur (Forssk) is much used in Ivory Coast and other places as medicine (Clark,1996). It is considered diuretic and aphrodisiac and is often used for sterility probably because of the numerous fruits. The root decoction is used as an enema and the latex of the young shoot is drunk as a cure for gonorrhea. A decoction of the leafy stem is drunk for dysentery and the tree is used for lumbago, headache and bronchial trouble (Nsi,2003).

The fresh leaves of Ficus Sur (Forssk) is used as vegetable, blood builder and medicine. It is used to treat diarrhea and anaemia as well as sexually transmitted diseases (Irvine,1961 and Wambebe,1998). The constituents responsible for the blood building ability and healing activities have not been extensively and exhaustively investigated and are the focus of this research.

2. Materials and Method

Collection of Sample

The plant material was collected from Umunze, headquarter of Orumba-South Local government area of Anambra State Nigeria. Umunze is located in the south-eastern part of Nigeria. Its geographical coordinates are 5o 58’ 0’’ North, 7o 13’ 0’’ East. The climate is tropical with considerable variation in the total annual rainfall from year to year with an annual mean rainfall of about 1824mm, average maximum temperature of 29oc and average minimum temperature of 23oc. It has a relative humidity of 80% at dawn (Ezenwaji et al.,2014)

Sample Preparation

The leaves of the plant was dried at room temperature for two weeks. It was pulverized with an electric blender and used for a series of test and analysis.

Preliminary Phytochemical Tests (Harbon,1996, Kannan et al., 2015, Sofowara, 1993 and Trease and Evans,1989)

Test for Flavonoids: Ammonium Solution Test and 1% Aluminium Chloride Test 10ml of ethyl acetate was added to about 0.2g of powdered plant material and was heated on a water bath for 3 minutes. The mixture was cooled, filtered and filtrate was used for the following tests:

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1) Ammonium Solution Test: About 4ml of filtrate was shaken with 1 ml of dilute ammonium solution. The layers were allowed to separate and the yellow color in the ammoniacal layer indicated the presence of flavonoids.
2) 1% Aluminium Chloride Solution Test: Another 4ml portion of the filtrate was shaken with 1 ml of 1% aluminium chloride solution. The layers were allowed to separate. A yellow colour in the aluminium chloride layer indicated the presence of flavonoids.

Test for Saponins: Frothing Test, Emulsion Test and Fehling's Test 20ml of water was added to 0.25g of the powder in 100ml beaker and boiled gently on a hot bath for 2 minutes. The mixture was filtered hot and allowed to cool and the filtrate used for the following tests:

Frothing Test: About 5 ml of the filtrate was diluted with 20ml of water and shaken vigorously. A stable froth upon standing indicated the presence of saponins.

Emulsion Test: To the frothing solution was added 2 drops of olive oil and the content shaken vigorously. The formation of emulsion indicated the presence of saponins.

Fehling Test: To 5ml of filtrate 5ml fehling solution was added and the content heated. A reddish precipitate indicated the presence of saponins. It was heated further with sulphuric acid and brick red precipitate appeared confirming the presence of saponins.

Test for Alkaloids: Dragendoff's Reagent, Meyer's Reagent and Wagner's Reagent. 20ml of 5% sulphuric acid in 50% ethanol was added to about 2g of the crude plant material and heated on a boiling water bath for 10 minutes, cooled and filtered. 2ml of the filtrate was tested with a few drops of Mayer's reagent (potassium mercuric iodide solution), Draggendoff's reagent (bismuth potassium iodide solution), Wagner's reagent (iodide in potassium iodide) and picric acid solution (1%). The remaining filtrate was placed in 100ml separatory funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portion of dilute sulphuric acid. The extracts were tested with a few drops of Mayer's reagent, Wagner's reagent, picric acid reagent and Draggendoff's reagent. Alkaloid gave milky precipitate with one drop of Mayer's reagent, reddish brown precipitate with one drop of Wagner's reagent and reddish precipitate with one drop of picric acid reagent. No precipitate was seen with Draggendoff's reagent but turbidity obtained.

Test for Tannins: Ferric Chloride Test. About 1g of powdered crude plant sample was boiled with 50ml of water, filtered and the filtrate was used for the following test:

Ferric Chloride Test: To 3ml of the filtrate two drops of ferric chloride were added. Presence of green precipitate indicated the presence of tannins.

Test for Resins: Precipitation Test and Colour Test

Precipitation Test: About 0.2g of the powdered material was extracted with 15ml of 96% ethanol. The alcoholic extract was then transferred into 20ml of distilled water in a beaker. A precipitate occurred which indicated the presence of resins.

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Colour Test: About 0.12g of the powdered leaves sample was extracted with chloroform and the extract was concentrated to dryness. The residue was redissolved in 3ml acetone and 3ml conc Hcl added. This mixture was heated in a water bath for 30 minutes, a pink colour which changed to magenta red indicated the presence of resin.

Test for Acidic Compounds: Litmus Paper Test. About 0.1g of crude plant sample was placed in a clear test tube and sufficient water was added. This was warmed in a hot water bath and then cooled. Apiece of water-wetted litmus paper was dipped into the filtrate and colour change on the litmus was observed to be nil.

Test for Protein:

Millon's Test: To a little portion of the filtrate in a test tube two drops of Millon's reagent were added. A white precipitate indicated the presence of protein.

Test for Reducing Sugar:
About 0.1g of the crude sample was shaken vigorously with 5 ml of distilled water and filtered. The filtrate was used for the following test:

1) Fehling Test: 1ml portion of the filtrate was added to equal volumes of fehling solutions A&B and boiled on water bath for few minutes. Presence of precipitate indicated the presence of reducing sugar.
2) b. Benedict Test: To 1ml portion of the filtrate was added 2ml of Benedict's reagent. The mixture was shaken, heated on a water bath for 5 minutes. Rusty brown precipitate showed the presence of reducing sugar.

Test for Oils:

1) 0.5 g of the sample was extracted with 5.0 ml of 90% alcohol and filtered. To the filtrate was added 3 drops of 5% ferric chloride solution, presence of green colour showed the presence of oil.
2) 0.5 g of sample was shaken in dilute NaOH (0.1ml) and filtered. To each of the filtrate was added 2 ml of dilute Hcl.

Presence of precipitate indicated the presence of oil.

Test for Carbohydrate:

1) Iodine Test

About 0.1g of the sample was mixed with a drop of iodine solution. A blue black colour indicated the presence of starch.

Extraction and Isolation of Alkaloids
12gof the sample was homogenized for 4minutes in methanol and water in the ratio of 4:1 by volume and then filtered. The filtrate was evaporated to one tenth of its volume and acidified with 2M H2SO4. After this chloroform (x3 volume) was added and the mixture transferred to a separatory funnel. Two layers were formed, chloroform extract and aqueous acid. The chloroform extract layer was evaporated and the moderately polar extract obtained which was likely terpenoid with phenolics was used for antimicrobial screening. The aqueous acid layer was basified to pH 10 with ammonium hydroxide and extracted with chloroform and methanol in the ratio 3:1 twice

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using a separatory funnel. This extract gave two layers, The chloroform methanol layer and aqueous basic layer. The aqueous basic layer was evaporated and extracted with methanol. This yielded a powdered alkaloid which weighed about 2.5g (Pavia,1982; Roger and Charles,1979).

Determination of Total Ash Content

2.0g of the powdered leaves was weighed and transferred into a weighed crucible. It was heated in a muffle furnace at 550oC for 4hours.The weight of the crucible containing ash was taken after cooling to the required temperature. The weight of the crucible containing ash was taken after cooling to the required temperature. The weight difference was expressed in percentage (Vermer,2003).

% Ash= (W2 -W1)/ (Sample weight) x100

Where; W1= Weight of crucible, W2 = Weight of crucible and ash.

Moisture Content Determination

2.0g of the fresh crude sample was weighed and transferred into a clean tray. It was dried at room temperature for about one week during which reweighing was done at every 24hours until a constant weight was obtained. The drop in weight was calculated and used for moisture content determination (Ibifoye,2006).

% Moisture content= (W2 -W1)/W1 x100

Where: W1 = Initial weight, W2 = Final weight

Stock Solutions of Alkaloid Extract

These were made using 50% acetone and sterile distilled water.2ml of each solvent was added to the alkaloid extract to form a stock solution ready for use

Antibacterial Test

Stock cultures of bacterial species namely, Streptococcus species, Staphylococcus aureus, Salmonella species, Klebsiella aerogens, Enterobacter aerogens, Pseudomonas pyocyania, Proteus vulgaris and Bacillus species were subcultured unto fresh nutrient agar and incubated at 37oC for 24hours.Young cultures of test organisms were obtained. A loopful of each test organism was emulsified in 5ml of normal saline to obtain a smooth suspension of the organism ready for use (Adebayo et al.,2009; Ahmad et al.,2016)

Antifungal Test

Stock cultures of Candida albican, Aspergillus flavus and Aspergillus niger were subcultured unto fresh saboraud dextrose agar and incubated at room temperature (28-30oC) for four days. Candida culture grew well within three days followed by others. A loopful of each test organism was emulsified in 5 ml sterile distilled water with the aid of glass beads. Any lump remaining was allowed to settle at the bottom of the bottles before use.

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Materials and media used for this test were sterilized before use. With the aid of sterile 6mm diameter agar puncher, four wells or holes of 6mm in diameter were made on each plate. The holes were sufficiently spaced and labelled accordingly to match with the extracts (Pelczar et al., 1993)

The punched plates were seeded with the test cultures of the organisms respectively using sterile swab sticks dipped in each suspension. Five minutes was allowed for the culture to dry on the agar. The labeled wells were then carefully filled with two drops (0.05cm3) of the different extracts. The plates were kept for thirty minutes on the bench for diffusion of extracts to take place. From the wells the extracts diffused into the surrounding medium and acted on the test organism. The plates were incubated with the base downward at 37oCfor 24hours for bacterial species and at room temperature (28 to 30oC) for 48 hours for fungal species. Zones of inhibition were observed, measured and recorded (Nsi, 2004).

Test for Vitamin C

20g of the powdered plant sample was extracted with 180ml of 0.4% oxalic acid. A dilute solution of 2,6-dichlorphenol indophenol (0.00019M) was used to titrate the ascorbic acid in the acid extract of the plant sample and the colour change was noted(Porgracz,1971).

Cocentration of vitamic C in the sample = 127.25mg/100g (0jukwu and Nwobi, 2017)

Test for Vitamin B

Extraction of the sample involved hot acid digestion in which the sample was heated in a 0.1M hydrochloric acid for about 15 minutes. It was then cooled and filtered. The filtrate was titrated against a standard solution of potassium hexacyanoferrate (iii) and the colour change was noted.

Determination of Nutrient Metals

Fe, Cu, Mg and Mn were determined by atomic absorption spectrophotometry (Ibitoye,2006). (Explain how they were determined or cite a reference of a previous work where the method was explain.

3. Results and Discussion

The phytochemical analysis of the powdered leaf sample reveals the presence of many metabolites like saponins, flavonoids, alkaloids, tannins, resins, reducing sugar, protein, oil and carbohydrate which are necessary for the functioning of human system (Nsi,2006).The results are in agreement with those obtained by Ahmad (2016). Saponins increase the rate of absorption by blood and flavonoids being derived from flavone which on hydrolysis yields salicylic acid are used to relieve pain and inflammation (Khedr et al., 2015). Tannins are used to control microorganisms, treat small wounds, catarrh, diarrhea and tan mucus layer to prevent bacterial action and chemical irritation (Finar,1977 and Harborne,1967).

Vitamin C which is very important in the formation of blood cell is seen to be contained by this plant. Also vitamin B which is necessary for the formation of blood is found to be present.

The instrumental analysis reveals the presence of iron, copper, manganese and magnesium. These elements play vital role in the formation of blood in human system.

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Furthermore, the antimicrobial screening done with the different extracts of this plant show that the extracts have inhibition effect on Enterobacter, Bacillus species, Pseudomonas pyocyania, Staphylococcus aureus, Aspergillus niger and Aspergillus flavus. This is comparable with the results obtained by Solomon-Wisdom, (2011). Thus the plant cannot only be used as a blood builder but as a medical treatment for ailments caused by bacteria and fungi.

The leaf extract of this plant can satisfactorily cure disease like anaemia, malnutrition, cold and supportive infections like boils, superficial infections such as skin pustules, conjunctivitis, septicaemia, intestinal diseases, food poisoning, surgical wounds and burns, acute bacterial endocarditis and bacteremia (Solomon-Wisdom,2011).

If this plant extract could be purified it could be quite close or even compete with the universal antibiotic, streptomycin (Solomon-wisdom et al., 2011).

4. Conclusion

The result obtained show that Ficus Sur (Forssk) has nutritional value and blood building ability. It is therefore recommended that this plant be included in the diet of people suffering from anaemia and also the dry leaves be taken as tea in food supplement. Apart from nutritional value, it has medicinal uses as shown by the antimicrobial screening. It is therefore advised that the plant be cultivated in our localities together with other vegetables.

Table 1: Phytochemical results.

Constituent	**Test**	**Observation**	**Inference**
Flavonoids	a) Dilute Ammonium solution test b) 1% Aluminium chloride test	Yellow colour observed Yellow colour observed	Present Present
Saponins	a) Frothing test b) Emulsion test c) Fehling's test	Persistent foaming Emulsion formed Red precipitate formed	Present Present Present
Alkaloids	a) Meyer's reagent b) Wagner's reagent c)Draggendoff's reagent	Milky precipitate formed Red precipitate formed Red precipitate formed	Present Present Present
Tannins	a) Ferric chloride test	Black precipitate formed	Present
Resins	a) Precipitation test b) Colour test	Caddy precipitate Pink colour which changed to magenta red	Present Present
Acidjc compound	a) Litmus paper test	No change of colour	Absent
Proieins	a) Millon's test b) Picric acid test	Deep red precipitate formed Yellow precipitate formed	Present Present
Reducing sugar	a) Fehling's test b) Benedict's t est	Red precipitate formed Rusty brown precipitate formed	Present Present

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Oil	a)5% Ferric chloride test b) 0.1M NaOH and 0.1M HCl test	Green colour observed Precipitate formed	Present Present
Carbohydrate	a) iodine test	Blue black colour observed	Present

Table 2: Result of Atomic Absorption Analysis.

Elements	Fe	Cu	Mg	Mn
Mg/100g	6.5	1.1	2.3	0.7

Table 3: Results of Antibacterial Activities of Extracts (Zones of Inhibition, ZOI in mm of diameter).

S/N	Solvent	Volume used in cm^3	Enterobacter aerogens (ZOI)	Proteus vulgaris (ZOI)	Bacillus species (ZOI)	Klebsiella aerogens (ZOI)	Pseudomonas Pyocyania (ZOI)	Staphylococcus aureus (ZOI)	Salmonella species (ZOI)	Streptococcus species (ZOI)
1	Chloroform	0.05	26	NA	14	NA	14	24	NA	NA
2	Chloroform methanol	0.05	24	NA	12	NA	18	26	NA	NA
3	Alkaloid 50% acetone	0.05	16	NA	10	NA	10	16	NA	NA
4	Alkaloid distilled water	0.05	14	NA	8	NA	7	14	NA	NA
5	Ethyl acetate	0.05	10	NA	NA	10	12	16	NA	NA
6	Control 50% acetone	0.05	NA	NA	NA	NA	NA	NA	NA	NA

NA =No Action.

Table 4: Result of Antifungal Activity of Extracts (Zones of Inhibition).

S/N	Solvent extracts	Volume used	Aspergillus niger	Aspergillus Flavus	Candida albican
1	Chloroform	0.05	10	8	NA
2	Chloroform methanol	0.05	8	8	NA
3	Alkaloid 50% acetone	0.05	6	5	NA
4	Ethyl acetate	0.05	7	6	NA
5	Control 50% acetone	0.05	NA	NA	NA

Table 5: Result of Proximate Analysis.

Proximate Analysis	Percentage
Moisture content	67.5
Ash content	65

Table 6: Result of Vitamin C Content

Concentration of vitamin c in the sample	127.25mg/100g

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