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Preliminary Phytochemical investigations and evaluation of Antimicrobial activity of n-hexane extract of the leaves of *Synclisia scabrida family menispermaceae*

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Abstract

The leaves of Synclisia scabrida were claimed to have antimicrobial properties. The leaves were used to treat wounds, running stomach, as well as fever. This investigation was carried out to ascertain the veracity of the claim. The leaves were collected and dried at ambient temperature and pulverized. 250gm of the powdered drug was extracted with 0.5 litre of n-hexane using the cold maceration technique for 24hours with occasional shaking. This was filtered and the process repeated using the marc. The combined filtrates were concentrated under reduced pressure with rotary evaporator. The preliminary phytochemical test were carried out using standard methods. The antimicrobial activity was evaluated using agar diffusion method. The leaves of synclisia scabrida exhibited antimicrobial property. Alkaloid, flavonoids, saponins, terpenoids and glycosides were found. Conclusion – the claim on the use of Synclisia sabrida appears to be obvious in line with the results of the investigation.

Keywords: Synclisia scabrida, agar diffusion, n-hexane, marc.

Introduction

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use[1]. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations[2]. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some case) and the cultural and spiritual point of view of the people of these countries[2].

In developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited[3]. Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase to 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used become necessary[4].

Before the era of Louis Pasteur (1822-1895), world renowned chemist and biologist who proved the germ theory of disease, the notion that tiny organisms could kill vastly larger ones (including human) seemed ridiculous to many people[5]. Nowadays, it has been accepted that infectious diseases are the number one causes of death worldwide, accounting for approximately one half of all deaths in tropical countries[6]. In fact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents.

The use of plant parts as a source of medicine to treat infectious diseases predates history[7]. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines to cure infections[8]. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control[9]. Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria. Even the world health organization is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs. Much of the exploration and utilization of natural

products as antimicrobials arise from microbial sources[10]. It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin. Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have also been a source of antibiotics. Examples of these are the bacteriostatic and antifugicidal properties of *Lichens,* the antibiotic action of allinine in *Allium sativum* (garlic), or the antimicrobial action berberines in goldenseal (*Hydrastis canadensis*). Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur. Plants based antimicrobials have enormous therapeutic potentia. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[11]. They are effective, yet gentle. Many plants have tropisms to specific organs or systems in the body. Phytomedicines usually have multiple effects on the body.Their actions often act beyond the symptomatic treatment of disease[12]. An example of this is *Hydrastis canadensis*. *Hydrastis* not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the spleen to release mediating compounds[13].

However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis. This work is therefore a preliminary work to prove that there is scientific evidence to the use of the root of Synclisia sabrida in the treatment of diseases.

The plant taxonomy goes thus; Domain: Eukaryota, Kingdom: Plantae, Subkingdom: Viridaeplantae, Phylum: Tracheophyta, Subphylum: Euphyllophytina*,* Infraphylum: Radiatopses, Class: Magnoliospida, Subclass: Ranunculidae, Super order: Ranunculanae, Order: Ranunculales, Family: Menispermaceae, Genus: Synclisia, Specific epithet: *scabscabrida* Miers.

Dioecious liana with twining stems up to 40 m long, with clear sap or sometimes white latex; bark dark brown; stems with long, stiff hairs. Leaves opposite, simple and entire; stipules absent; petiole 2–4 cm long, swollen at base and apex, reddish hairy; blade lanceolate-ovate, 5–12(–20) cm × (4–)7–9 cm, base cordate, apex acuminate, upper surface thinly hairy, lower surface densely hairy, pinnately veined, but with 1–2 pairs of basal veins and 4–5 pairs of lateral veins. Flowers axillary, solitary or in pairs, unisexual; pedicel slender, c. 2 cm long, densely hairy, with 2 minute bracts; sepals 9, 6 outer ones bract-like, linear-lanceolate, 1–2 mm × 0.5–1 mm, apex acute, densely hairy outside, 3 inner ones linear-lanceolate, basally merged into an urn-shaped tube, 7–8 mm × c. 1.5 mm, fleshy; petals 6, c. 0.5 mm long, rounded, fleshy, glabrous; male flowers with 6(–9) stamens, basally fused, 3 outer ones c. 2 mm long, 3 inner ones c. 3 mm long, filaments slender at apex, inner ones bending outwards, outer ones bending inwards; female flowers with spoon-shaped staminodes c. 3 mm long, ovary superior, composed of 15–30 laterally compressed carpels, with long, stiff hairs, styles lateral, slender, c. 3 mm long, stigma small, triangular. Fruit composed of a dense head of obovoid-ellipsoid drupes 12–17 mm × 8–9 mm, orange, densely reddish hairy, with swollen apex, stone bony, fine-hairy outside, 1-seeded. Seed horseshoe-shaped, 1–1.5 cm long, cotyledons very unequal.

Synclisia scabrida occurs from Nigeria east to the Central African Republic and south to DR Congo and Angola. *Synclisia scabrida* occurs in rainforest, including secondary forest, at low and medium altitudes. Plants are only collected from the wild. Leaves for fodder can be harvested at any time of the year. Individual leaves or the whole liana are cut and fed fresh or dried. *Synclisia scabrida* has a wide distribution and also occurs in secondary vegetation; there are no indications that it is in danger of genetic erosion.

Ethnobotanical Uses of *Synclisia scabrida:* In Nigeria and Cameroon an alcoholic leaf decoction is drunk to treat gastric ulcers. In Nigeria the root soaked in alcohol or macerated in boiling water is taken to treat malaria, to prevent threatened abortion and as a common medicine to calm patients with mental disorders, e.g. psychoses. In Gabon the bitter root, sometimes mixed with stem bark of *Garcinia klainii* Pierre ex Engl., is put in palm wine, which is drunk to treat venereal diseases and as an aphrodisiac and also to treat prostate problems, asthma and hernia. In Congo pregnant women may tie a piece of liana around the waist to avoid spontaneous abortion. In Gabon a root decoction is used in trial by ordeal ceremonies; when it causes constipation one is innocent, when it causes diarrhoea, one is guilty. The root bark contains a yellow dye of unrecorded use. The leaves are used as protein-rich fodder for ruminants.

Material and Methods

The chemicals used for extraction processes include n-hexane, Dimethyl sulphoxide (KERMEL), Nutrient Agar (ANTEC Diagnostic products, United Kingdom) Sabouraud dextrose agar (Fluka Biochemicha, India).

The reagents used were – concentrated sulfuric acid, naphthol solution in ethanol (Molisch reagents) picric acid, ammonium solution, nitric acid, aluminum chloride solution, Fehling solution A and B, Wagner's reagents (iodine and potassium iodide), Hager's reagent (saturated solution of picric acid). The microorganisms used were both bacteria and fungi obtained from laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Agulu campus.

The organisms include bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosae, Escherichia coli, Bacillus subtilis*, *Salmonella typhi*) and *Aspergillus niger* and *Candida albicans* were the two fungi used.

Equipments used include**;** Weighing Balance [Scout pro u401 made in China], Beakers, measuring cylinder, test tubes**,**

incubators (GentLab UK), autoclave, test tubes, test tube racks, syringes and needle, Pasteur's pipette, conical flask, glass rod, inoculation loop, Tripod stand, filter paper (Whatman No 1), Mortar and pestle , water bath, muslin- cloth, reagent bottles, Bunsen burner, and permanent marker.

The fresh leaves of *Synclisia scabrida* were obtained from Ogidi in Anambra State in November 2011. The plant was identified by Mr Ozioko, a Taxonomist.The leaves were air dried in the Pharmacognosy Laboratory and then pulverized to produce 250g of powdered plant leaf.

Extraction Process: Methodology – The leaves was collected and dried at ambient temperature and pulverized. 250gm of the powdered drug was extracted with 0.5 litre of n-hexane using the cold maceration technique for 24hours with occational shaking. This was filtered and the process repeated with the marc. The combined filtrates were concentrated under reduced pressure with rotary evaporator. Standard screening tests were carried out on both powdered leave and crude extract for various phytochemical constituents. The procedure used was obtained from Trease and Evans.

Antimicrobial assay; 24hour Cultures of five human pathogenic bacteria made up of both gram positive (*S. aureus,* and *B.subtilis*) and and gram negative (*P. aeruginosa, E. coli* and *S. typhi*) bacteria were used for the *in-vitro* antibacterial assay. For the antifungal assay, two fungi were utilized for the studies and these were made up of *Aspergillus niger* and *Candida albicans*. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka.

Preparation of media: Nutrient broth, nutrient agar, sabouraud dextrose agar (SDA) were used in the assays. Dimethylsulphoxide (DMSO) was used in solublising the extracts and drugs and as a negative control in the study. The media were prepared by dispersing the weighed amount in water and then sterilizing them with autoclave. The plates of nutrient agar were poured and allowed to solidify after the appropriate organisms were seeded.

Antimicrobial agents: perfloxacin, 5ug/ml (Mecure indusrial ltd lagos Nigeria.); Clotrimazole cream, 1mg/ml (Drug field, Nigeria) were included in the study as standard reference drugs.

Antimicrobial activity determination: An overnight broth culture used to obtain 0.5 Marcfarland standard of bacterium was used to seed sterile molten nutrient agar medium maintained at 45°C. Sabouraud dextrose agar plate was similarly seeded with fungi. Seven holes (6mm) respectively, were bored in each of the plates (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified; 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml of extract were prepared in dimethylsulphoxide (DMSO) by preparing a stock solution and carrying out double fold dilutions on it. And with the aid of a Syringe, the wells were filled with 0.25 ml (5drops) of different dilutions of the extract while the centre wells were filled with 20µg/ml and 1 mg/ml of ampicillin and clotrimazole cream for bacteria and fungi respectively (also dissolved in DMSO). Diameters of zones of inhibition were determined after incubating plates at 37°C for 24h for the bacteria and at 25°C for 72 hours for fungi respectively. Dimethylsulphoxide was used as negative control while perfloxacin and clotrimazole cream were used as positive control.

Results and Discussion

The results of phytochemical screening showed increased presence of glycosides, simple sugar and abundance of flavonoids, and glycosides, moderate quantity of protein, little quantity of steroids, phenols and tarnins. There was however, an absence of volatile oils. The extract displayed various activities against bacteria inhibiting it at various concentrations ranging from 400 to 50mg/ml. The n-hexane extract, however, gave activity *Staph aureus*. IZD's of 16, 12, 8 and 4mm were recorded for concentrations of; 400, 200, 100, 50mg/ml respectively. There was no activity however for *Pseudomonas aureginosa* and bacillus subtilis and almost same for *Salmonella typhi*.

Table-1
Result of Phytochemical Screening of *Synclisia scabrida* and yields of extracts

Test for	Prescence
Phenols	+
Glycosides	+
Sarponins	+
Tarnins	+
Alkaloids	_
Volatile oils	_
Sugars	+
Flavonoids	+
Proteins	+
Steroids	+

Key; - = not detectable; + = low concentration; ++= medium concentration; +++= High concentration

The extract of *Synclisia scabrida* showed varied activity against the different pathogens used. This could be attributed to the presence of some active principles (secondary metabolites); phenols, glycosides, sarponins, reducing sugars, flavonoids, and proteins, as determined by the phytochemical tests conducted. Some of these active principles have been reported to have activity against micro-organisms.

As shown the n-Hexane extract exhibited bacteriostatic activities, best against *S.aureus* with IZD's ranging from 4-16mm at concentrations ranging from 100-400mg/ml., it also showed activity against *S.typhi* and *B.subt*. but no action against Ecoli and P.aeruginosa.

Table-2
Antibacterial activity n-hexane extract

N -hexane	Inhibition Zone Diameter For Bacteria for Different concentrations of Extracts (mm)						
	400	**200**	**100**	**50**	**25**	**12.5 mg/mL**	**Perfloxacin (5ug)**
S. aureus	16	12	8.0	4.0	-	-	25
P. aeruginosa	-	-	-	-	-	-	14
E. coli	-	-	-	-	-	-	11
B. subtilis	6.0	4.0	-	-	-	-	10
S. typhi	2.0	-	-	-	-	-	9

Table-3
Analysis of the Result

Bacteria	**n-Hexane Extract**
S.aureus	50
P.aureginosa	-
S.typhi	400
E.coli	-
B.subtiis	200

Minimum inhibitory zone diameter

Conclusion

From the result the plant was active on some bacteria studied. However, further tests are recommended to unravel more activity of the plant.

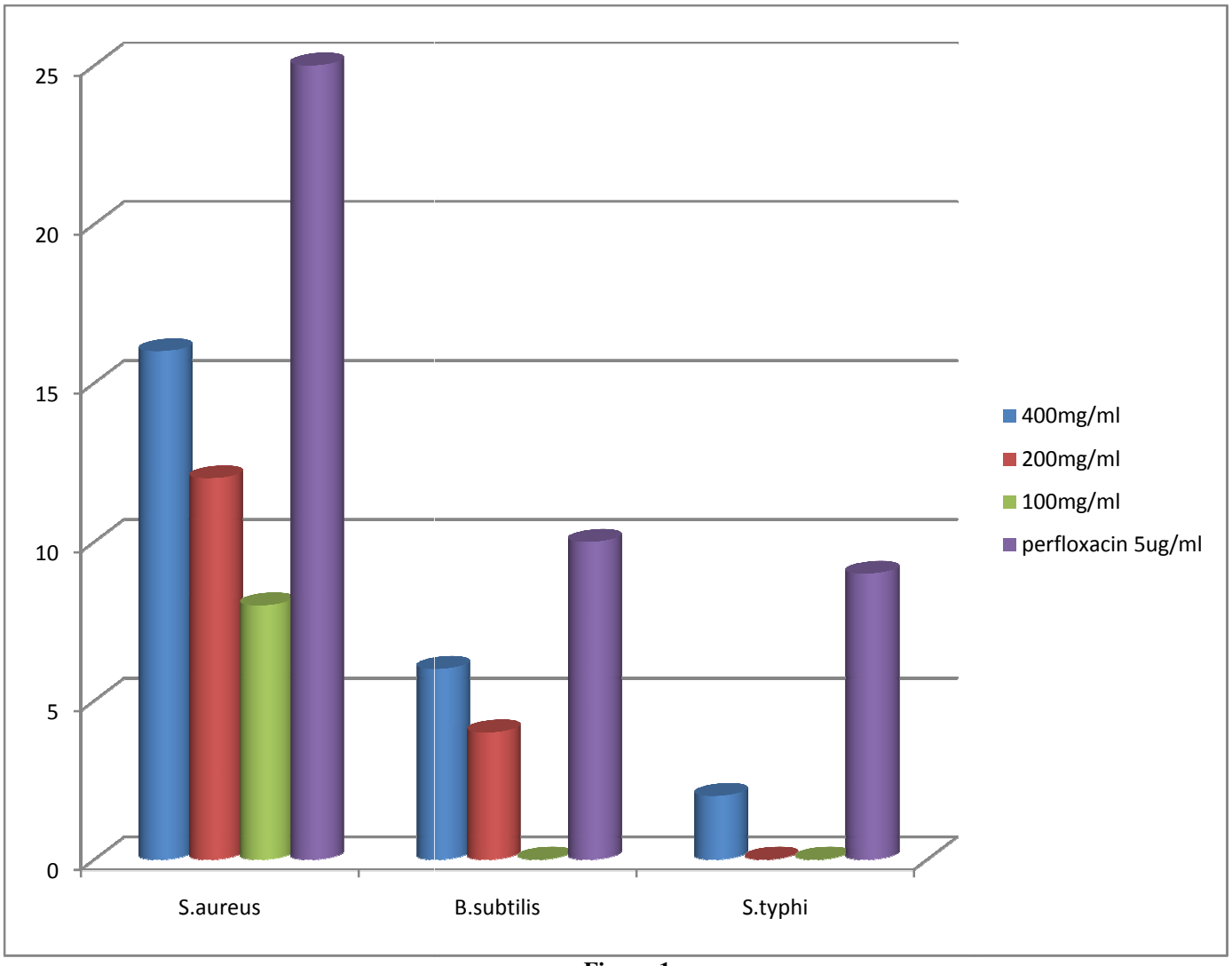

Figure-1
Activity data of N-hexane extract on susceptible pathogens compared with activity of standard drug

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