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Antimicrobial activity and Chemical Composition of the flowers of *Aspilia africana*

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Abstract

Aspilia africana flower obtained in Kafanchan Kaduna state, Nigeria from the wild was extracted using methanol as solvent in order to investigate its phytochemical, antimicrobial, toxicity and antioxidant characteristics. This is to investigate an alternative source of natural colourants which is bioactive. The crude extract has inhibitory activity on *Candida albicans, Staphyllococcus aureus, Escherichia coli, Bascillus subtilis, Pseudomonas aeriginosa* and *Aspergillus flavus.* The extract has low toxicity with LD_{50} 6.5g/kg body weight. LC_{50} 2.10 and antioxidant activity of 96.8 which compare favourably with a standard antioxidant, ascorbic acid.

Keywords: Antimicrobial, antioxidant, phytochemistry, toxicity.

1.Introduction

Aspilia africana belongs to the family compositae. It is a semi woody herb from a perennial stock up to 2m high. It occurs in wasteland Savannah forested zones and occurs across Tropical Africa (Dalziel, 1955). It is weedy, grazed by sheep and cattle and is used in west Sahara as food for rabbits and hares. It has high crude protein content (Burkill, 1999). It is used in herbal medicine to treat infection of bacterial origin such as gonorrhea, stomach trouble, and corneal opacity. It is use as haemostatic agent (Dalziel, 1955). Fresh leaves are used on cuts, sores and wounds for healing. A decoction is used as pulmonary haemorrhages and haemostasis due to vasoconstriction (Irvine, 1961). In Tanganyika a root decoction is used for tuberculosis and in Ghana leaves are made into cough syrups for children. In Uganda, leaf decoction is used for gonorrhea and oils of seed contain fatty acids.

Investigation on the toxic effects of methanolic extracts of *Aspilia africana* leaf on the estrous cycle and uterine tissues of wistar rats, the study reveals a dose dependent toxicity where estrous cycle were significantly reduced that is it possess negative effect on estrous cycle and histo-architecture on uterus of female rats suggesting negative influences on reproductive health of the animals. Eweka (2007) reported a histological studies of the teratogenic effects of oral administration of *Aspilia africana* leaf extract on the developing liver of neonatal wistar rats. It is observed that there is a direct cytotoxic effects of aqueous extract of *Aspilia africana* during pregnancy. It highlight possible anomalities that could result in newborn when a pregnant animal is exposed to aqueous extracts of *Aspilia africana* in the first few days of pregnancy. Okoli *et al.,* (2007) showed that *Aspilia africana* leaves possess constituent capable of arresting wound bleeding, inhibiting growth of microbial wound contaminants and accelerating wound healing which suggest good potential for use in wound care. It is used traditionally to stop bleeding in wounds, clean the surfaces of sores, in treatment of rheumatic pains, bee and scorpion stings and for removal of opacities and foreign bodies in eyes.

2. Experimental Procedure

2.2 Preparation of the plant

Aspilia africana flowers were obtained in Kafanchan, Kaduna state, Nigeria and identified in National Institute for Pharmaceutical Research and development, Idu, Abuja. Samples were cleaned and air-dried to constant weight for some weeks and stored in a desiccator before analysis.

2.3 Extraction

The dried flowers were extracted with methanol in a soxhlet extractor as described by Furniss *et al.,* (1978) for 3hrs at a solute solvent ratio of 1:10w/w. The solvent was then removed from the extract by using a rotatory evaporator and extract transferred to an evaporating dish and dried on a water bath. Extract was then stored in a refrigerator until needed for analysis.

2.4 Phytochemical studies
Phytochemical screening for major constituents presents in the flowers were carried out using standard methods (Trease and Evans, 1989; Harbone, 1998; Odebiyi and Sofowora, 1993).

2.5 Antimicrobial Screening
The Bauer and Kirby (1996) disc diffusion method was used to determine the antimicrobial activity of the plant extract on these microorganisms: *Staphyllococcus aureus, Bascillus subtilis, Escherichia coli, Pseudomona aeriginosa, Candida albicans, Salmonella typhi, Aspergillus flavus*. The zones of inhibition and minimum inhibitory concentrations at concentrations of 2.0, 1.0, 0.5, 0.25, and 0.125mg/cm^3 of extract respectively were obtained using the interpretation chart of Kirby-Bauer (1996). The controls were maintained in dimethylsulphoxide.

2.6 Toxicity Test
The test was carried out on the extracts of flowers using Brine shrimp lethality bioassay (Meyer, *et al.,* 1982). The data obtained were subjected to analysis to determine the LC_{50}.

2.7 Acute toxicity and lethality (LD_{50}) test
The LD_{50} of the methanol extract was determined on albino mice using the method of Lorke (1983).

2.8 Antioxidant Analysis
Antioxidant, free radical scavenging assay of the flower extracts at 250µg/cm^3 was evaluated using the 1,1–diphenyl–2–picryl hydrazyl (DPPH) assay at 518nm (Mensor *et al.,* 2001; Aderogba *et al.,* 2004). The results are expressed as mean± standard error of the mean (SEM).

3. Results and Discussions
3.1 Phytochemical and Antimicrobial Characteristics
Aspilia africana flower extracts contains the following phytochemicals: alkaloid, tannin, saponin, flavonoid, phenol and glycosides. The extracts inhibit the growth of *Staphylococcus aureus*, *Bascillus subtilis, Escherichia coli* and *Salmonella typhi* at the minimum inhibitory concentration of 2.0mg/cm^3 respectively while *Pseudomonas aeriginosa, Candida* albicans, and *Aspergillus flavus* have minimum inhibitory concentration of 1.0mg/cm^3 respectively. These phytochemical are secondary metabolites of natural products which are bioactive. Phytochemicals contribute to optimal health and lack of it leads to disorders called phytochemical deficiency disorders (Wahlqvist, 1997) such as cardiovascular disorder due to deficiency of phytochemicals which acts as antioxidants. Flavonoids display antiviral and antioxidant activity. Tannins hasten the healing of wounds and inflamed mucous membrane as they react with proteins forming water soluble copolymer therefore the wounds are free from attack of parasitic fungi, insects and yeasts infections.
Tannins are used in tanning hides and skin as it reacts with proteins and transforms animal skin to leather. Phenols, tannins and flavonoids are the major phytochemicals associated with antioxidant activity of medicinal plants which prevent oxidative cell damage and protects against allergies, inflammations, micro-organisms, ulcers, viruses and tumours, (Okwu and Omodamiro, 2005; Tirillini, 2000; Okunade, 2002).
Tannins and saponins regulates, protects and kills cancer cells such as cancer of prostrate, testicular cancer and improves semen quality in men. It also prevents breast cancer and cystic ovaries in women.
Saponins are characterized by its foaming property and therefore has great potentials as foaming agents, emulsifier in beverages, photographic emulsions, detergents, drugs, cosmetics and shampoo productions (Oakenful and Sidhu, 1990). Alkaloids are analgesics, antispasmotic for the treatment of hypertension, metal disorders and for pupil dilation (Trease and Evans, 1998; Okwu and Okwu, 2004).
The antimicrobial activity of extracts of *Aspilia africana* flowers are broad spectrum since gram positive and gram negative bacteria were sensitive to it. These bacteria have been implicated in diarrhea, oral and dental infection, wound sepsis and dysentery (Hugo and Russel, 1983). This flower contains great antifungal agent due to susceptibility of *C. albicans* and *A. flavus* at diameter of zones of inhibition of 20mm and 17mm respectively. *S. aureus, B. subtilis, P. aeriginosa* and *E. coli* shows zones of inhibition of 18mm, 15mm, 16mm and 14mm respectively.
According to Ibeh and Uraih (2003), an inhibition zone diameter of 10mm or less indicates that the organism is resistant; 11-15mm indicates an intermediate (mild) effect while 16mm and above indicates that the organism is susceptible to the extract which means that the extract has high antimicrobial effect. Therefore, *Aspilia africana* flower extract show high antimicrobial activity on *S. aureus, B. subtilis* and *P. aeriginosa* but mild effect on *E. coli.*

The antimicrobial action of flowers show ethnopharmaceutical claim of killing dysentery, wound sepsis and bacteria infection. Therefore, the presence of alkaloids, saponin, tannin and flavonoid is responsible for the efficacy of *Aspilia africana* flower extracts.

3.2 Toxicity and antioxidant activity

The acute toxicity studies revealed an oral dose LD_{50} of 6.5g/kg body weight and toxicity based on brine shrimp lethality bioassay, LC_{50} 2.10.

The antioxidant activity of the flower extract, % AA of 96.80% compared favourably with a standard antioxidant, ascorbic acid, with % AA of 93.7% which is used as a control in this research. The acute toxicity test of the extract suggests a remote risk of acute intoxication. This implied high degree of relative safety of the flower extract when administered as drugs. The low LC_{50} of the flower extract suggest that it is very toxic when applied to microorganisms that is very efficacious when applied as drugs. This implied that the extract could be formulated as drugs that could be used in controlling diseases caused by these micro-organisms which show sensitivity to it. The antioxidant activity shows that there are antioxidants which scavenge free radicals in the human system (Quettier-Dellen, 2000; Del Rio *et al.,* 1999) thereby promoting health of an individual.

4. Conclusion

Findings from this research confirms that the traditional medicinal use of *Aspilia africana* flower extract as a treatment of dysentery, wound sepsis, gonorrhea, stomach trouble, corneal opacity and as analgesics is justified and should be encouraged. The sensitivity of microorganisms to extract, antioxidant, phytochemical characteristics and toxicity of the extract give promise to their potential use in treatment of microbially induced disease conditions.

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